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(54) Title: ASSAYS FOR AGONISTS, AGONISTS AND INVERSE AGONISTS OF MELANIN CONCENTRATING HORMONE (MCH) BINDING TO THE SOMATOSTATIN–LIKE RECEPTOR (SLC–1)

(57) Abstract

The present invention is directed to assays that can be used to screen for compounds that act as agonists or antagonists of melanin concentrating hormone (MCH). The assays are based upon the binding of MCH to the SLC–1 receptor.

ASSAYS FOR AGONISTS, AGONISTS AND INVERSE AGONISTS OF MELANIN CONCENTRATING HORMONE (MCH) BINDING TO THE SOMATOSTATIN-LIKE RECEPTOR (SLC-1)

Field of the Invention

The present invention is directed to assay methods that can be used to determine whether a test compound can be used to modulate the binding of MCH to the SLC-1 receptor. Compounds identified as being effective modulators have potential use as therapeutic agents in treating obesity and eating disorders.

Background of the Invention

A. Melanin Concentrating Hormone

Melanin concentrating hormone (MCH) is a cyclic peptide that was first isolated from fish over 15 years ago. In mammals, MCH gene expression is localized to the ventral aspect of the zona inserta and the lateral hypothalamic area (Breton, et al., Mol. Cell. Neurosci. 4:271-283 (1993)). The latter region of the brain is associated with the control of behaviors such as eating and drinking, with arousal and with motor activity (Baker, Trends Endocrinol. Metab. 5:120-126 (1994)). Although the biological activity of MCH in mammals has not been fully defined, recent work has indicated that it promotes eating and weight gain (U.S. 5,849,708). Thus, MCH and its agonists have been proposed as a treatment for anorexia nervosa and weight loss due to AIDS, renal disease, or chemotherapy. Similarly, antagonists of MCH can be used as a treatment for obesity and other disorders characterized by compulsive eating and excessive body weight.

Although MCH has been known for over two decades, its specific receptor has not been structurally characterized and cloned. This has limited the ability to search for therapeutic agents that act by mimicking or inhibiting MCH.

B. G Protein-Coupled Receptors

G protein coupled receptors (GPCRs) constitute a family of proteins sharing a common structural organization characterized by an extracellular N-terminal end, seven hydrophobic alpha helices putatively constituting transmembrane domains and an intracellular C-terminal domain. GPCRs bind a wide variety of ligands that trigger intracellular signals

through the activation of transducing G proteins (Caron, *et al.*, *Rec. Prog. Horm. Res.* *48*:277-290 (1993); Freedman, *et al.*, *Rec. Prog. Horm. Res.* *51*:319-353 (1996)).

More than 300 GPCRs have been cloned thus far and it is generally assumed that there exist well over 1,000 such receptors. Roughly 50-60% of all clinically relevant drugs act by modulating the functions of various GPCRs (Gudermann, *et al.*, *J. Mol. Med.* *73*:51-63 (1995)). Many of the clinically relevant receptors are located in the central nervous system.

Among the GPCRs that have been identified and cloned is a gene that encodes a protein homologous to the receptors of the somatostatin family. Kolakowski called this receptor SLC-1 and described the structure of the gene as it exists in humans (*FEBS Lett.* *398*:253-258, (1996)). A rat counterpart of SLC-1 was found to be essentially identical and was described by Lakaye, *et al.* (*Biochim. Biophys. Acta. 1401*:216-220 (1998)). Based upon the location of cells expressing SLC-1 mRNA, it was proposed that the receptor plays a role in functions such as emotion, memory and sensory perception. However, the endogenous ligand of this receptor has not previously been identified.

Summary of the Invention

The present invention is based upon the discovery that MCH serves as a ligand for the SLC-1 receptor. Recombinant cells expressing either rat or human SLC-1 can be used in conjunction with MCH in screening assays designed to identify agonists and antagonists. Thus, in its first aspect, the invention is directed to a method of assaying a test compound for its ability to bind to the SLC-1 receptor. This is accomplished by incubating cells expressing the receptor gene with MCH and test compound. The extent to which the binding of MCH is displaced is then determined. Radioligand assays or enzyme-linked immunosorbent assays may be performed in which either MCH or the test compound is detectably labeled. Although any cell expressing SLC-1 may be used, a recombinant cell expressing a heterologous SLC-1 gene from either the rat or human is preferred. The term "heterologous" as used herein refers to any SLC-1 gene transfected into a cell, *i.e.*, the term refers to any non-endogenous SLC-1.

The invention is also encompasses methods of determining if a test compound is an agonist, antagonist, or inverse agonist of MCH binding based upon a functional assay. One way to carry out such assays is to incubate a cell expressing SLC-1 with the test compound

and to then determine whether intracellular adenyl cyclase activity or intracellular calcium concentration changes. Results should typically be compared with those obtained when incubations are performed in a similar manner but in the absence of test compound. In general, functional assays of this type will be performed in conjunction with binding assays of the sort described above. The preferred cell for use in the assays is a recombinant cell that has been transformed with a heterologous SLC-1 gene. Test compounds that act as agonists should produce an increase or decrease in adenyl cyclase activity or increase in intracellular levels of calcium. Inverse agonists may reduce adenyl cyclase activity or intracellular calcium levels, particularly if assays are performed in the presence of a fixed amount of MCH. Antagonists, should block the binding of MCH to receptor but not produce the opposite reponse in terms of adenyl cyclase activity or intracellular calcium that is the hallmark of an inverse agonist.

Detailed Description of the Invention

The present invention is directed to assays that can be used to screen compounds for their ability to modulate the binding of MCH to the SLC-1 receptor. Any form of MCH that has been reported may be used, but the preferred peptide is 19 amino acids in length and has the sequence: Asp-Phe-Asp-Met-Leu-Arg-Cys-Met-Leu-Gly-Arg-Val-Tyr-Arg-Pro-Cys-Trp-Gln-Val (SEQ ID NO:1). The peptide assumes a cyclic conformation as the result of a disulfide between the two cysteines. This peptide may be obtained commercially (Sigma, St. Louis, MO) or can be synthesized using standard methodology well known in the art. The peptide may be detectably labeled with radioisotopes such as ^{125}I or, alternatively, fluorescent or chemiluminescent labels can be incorporated. Also, the peptide can be joined to enzymes that are readily detectable such as horseradish peroxidase.

The SLC-1 receptor may be cloned from human cells using the procedure described by Kolakowski, et al. (*FEBS Lett. 398*:253-258 (1996)) or from rat cells using the procedure described by Lakaye, et al. (*Biochim. Biophys. Acta. 1401*:216-220 (1998)). The Examples section provides a detailed description of a procedure that may be used in cloning SLC-1 which, is also referred to herein as clone 1-18. Once obtained, the SLC-1 sequence should be incorporated into an expression vector with a promoter active in mammalian cells (Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Press (1989)). Examples of promoters that may be used include that of the mouse metallothionein I gene

(Hamer, *et al.*, *J. Mol. Appl. Gen.* *1*:273-288 (1982)); the immediate-early and TK promoter of herpes virus (Yao, *et al.*, *J. Virol.* *69*:6249-6258 (1995); McKnight, *Cell* *31*:355-365 (1982)); the SV 40 early promoter (Benoist, *et al.*, *Nature* *290*:304-310 (1981)); and, the CMV promoter (Boshart, *et al.*, *Cell* *41*:521-530 (1985)). Vectors may also include enhancers and other regulatory elements.

Once expression vectors have been constructed, they can be introduced into a mammalian cell line by methods such as calcium phosphate precipitation, microinjection, electroporation, liposomal transfer, viral transfer or particle mediated gene transfer. Although other mammalian cells may be used, HEK-293 cells have been found to give successful results and a procedure for expressing SLC-1 in these cells is described in the Examples section. Standard procedures for selecting cells and for assaying them for the expression of SLC-1 (*e.g.*, by Northern analysis) may be performed.

Once the MCH peptide and cells producing the SLC-1 receptor have been obtained, assays may be performed to determine whether test compounds have any effect on binding. A wide variety of different types of assays can be performed using standard methods well known in the art. For example, in radioligand binding assays, cells expressing SLC-1 are incubated with MCH and with a compound being tested for binding activity. The preferred source of SLC-1 is recombinantly transformed HEK-293 cells. Other cells may also be used provided they do not express other proteins that strongly bind MCH. This can easily be determined by performing binding assays on cells transformed with SLC-1 and comparing the results obtained with those obtained using their untransformed counterparts.

Assays may be performed using either intact cells or with membranes prepared from the cells (*see e.g.*, Wang, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* *90*:10230-10234 (1993)). As suggested above, the membranes, or cells, are incubated with MCH and with a preparation of the compound being tested. After binding is complete, receptor is separated from the solution containing ligand and test compound, *e.g.*, by filtration, and the amount of binding that has occurred is determined. Preferably, the ligand used is detectably labeled with a radioisotope such as [125]I. However, if desired, other types of labels can also be used. Among the most commonly used fluorescent labeling compounds are fluorescein, isothiocynate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin *o*-phthaldehyde and fluorescamine. Useful

chemiluminescent compounds include luminol, isoluminol, theromatic of acridinium ester, imidazole, acridinium salt, and oxalate ester.

Nonspecific binding may be determined by carrying out the binding reaction in the presence of a large excess of unlabeled ligand. For example, labeled MCH may be incubated with receptor and test compound in the presence of a thousandfold excess of unlabeled MCH. Nonspecific binding should be subtracted from total binding, i.e., binding in the absence of unlabeled ligand, to arrive at the specific binding for each sample tested. Other steps such as washing, stirring, shaking, filtering and the like may be included in the assays as necessary. Typically, wash steps are included after the separation of membrane-bound ligand from ligand remaining in solution and prior to quantitation of the amount of ligand bound, e.g., by counting radioactive isotope. The specific binding obtained in the presence of test compound is compared with that obtained in the presence of labeled ligand alone to determine the extent to which the test compound has displaced receptor binding.

In performing binding assays, care must be taken to avoid artifacts which may make it appear that a test compound is interacting with receptor when, in fact, binding is being inhibited by some other mechanism. For example, the compound being tested should be in a buffer which does not itself substantially inhibit the binding of MCH and should, preferably, be tested at several different concentrations. Preparations of test compound should also be examined for proteolytic activity and it is desirable that antiproteases be included in assays. Finally, it is highly desirable that compounds identified as displacing the binding of MCH be reexamined in a concentration range sufficient to perform a Scatchard analysis on the results. This type of analysis is well known in the art and can be used for determining the affinity of a test compound for receptor (see e.g., Ausubel, et al., Current Protocols and Molecular Biology, 11.2.1-11.2.19 (1993); Laboratory Techniques in Biochemistry and Molecular Biology, Work, et al., Ed. N.Y. (1978)). Computer programs may be used to help in the analysis of results (e.g., Munson, P., Methods Enzymol. 92:543-577 (1983)).

Depending upon their effect on the activity of the receptor, agents that inhibit the binding of MCH to receptor may be either agonists or antagonists. Activation of receptor may be monitored using a number of different methods. For example, adenyl cyclase assays may be performed by growing cells in wells of a microtiter plate and then incubating the wells in

the presence or absence of test compound. cAMP may then be extracted in ethanol, lyophilized and resuspended in assay buffer. Assay of cAMP thus recovered can be carried out using any method for determining cAMP concentration. Typically, adenyl cyclase assays will be performed separately from binding assays, but it may also be possible to perform
5   binding and adenyl cyclase assays on a single preparation of cells.

Activation of receptor may also be determined based upon a measurement of intracellular calcium concentration. For example, transformed HEK-293 cells may be grown on glass cover slides to confluence. After rinsing, they may be incubated in the presence of an agent such as Fluo-3 or FURA-2 AM (Molecular Probe F-1221). After rinsing and further
10   incubation, calcium displacement may be measured using a photometer. Other types of assays for determining intracellular calcium concentrations are well known in the art and may also be employed.

Assays that measure the intrinsic activity of the receptor, such as those based upon inositol phosphate measurement, may be used in order to determine the activity of inverse
15   agonists. Unlike antagonists which block the activity of agonists but produce no activity of their own, inverse agonists produce a biological response diametrically opposed to the response produced by an agonist. For example, if an agonist promoted an increase in intracellular calcium, an inverse agonist would decrease intracellular calium levels.

The radioligand and cell activation assays discussed above merely provide examples
20   of the types of assays that can be used for determining whether a particular test compound alters the binding of MCH to the SLC-1 receptor and acts as an agonist or antagonist. There are many variations on these assays that are compatible with the present invention. Such assays may involve the use of labeled antibodies as a means for detecting MCH that has bound to receptor or may take the form of the fluorescent imaging plate reader assays
25   described in the Examples section herein.

Examples

I.   Methods

Preparation of Clone 1-18

A PCR-based strategy was used to clone the rat 1-18 gene (SLC-1). Rat spinal cord
30   mRNA was isolated using the FastTrackÔ kit (InVitrogen, San Diego, Ca). The templates for

PCR amplification were synthesized using GeneAmp RNA PCR kits (N808-0017 Perkin Elmer) with 200 ng of the rat spinal dorsal horn polyA+ RNA and were amplified using the following primers:

TM3-5:   5'-G(C or T)G(A or C)(C or G)(A or G)(C or G)(C or T)ITIGA(C or T)
5   CGCTA-3' (SEQ ID NO:2)

TM7-5:   5'-AAGC(C or T)(A or G)TA(G or T)AI(A or C or G)AI(A or C)GG(A or
          G)TT-3' (SEQ ID NO:3).

The reaction mixture contained 200 pmoles of each of the TM3-5 and TM7-5 primers and 2.5 units of Taq DNA polymerase in 50 mM KCl, 1.5 mM MgCl$_2$, 10 mM Tris(HCl), 200 mM
10   dNTPs, pH 9.0. The reaction tubes were heated at 95 °C for one minute and subjected to 39 cycles of denaturation (95 °C / 1 min), annealing (42 °C / 1 min)and extension (72 °C / 1 min). The amplified fragments were analyzed and size fractionated on a 1% agarose gel. Fragments between 500 bp and 800 bp were excised from the gel, purified using the Sephaglas BandPrepÔ kit from Pharmacia (cat# 27-9285-01), and subcloned into the pGEM-T vector
15   from Promega (cat# A3600). Recombinant pGEM-T clones were selected randomly and plasmid DNA was prepared using the alkaline lysis method starting with 2 ml of bacterial culture. The Sanger dideoxy nucleotide chain termination method was used to sequence the DNA from these clones, with the T7 sequencing kit from Pharmacia (cat# 27-1682-01). The insert DNA fragment of the clone pGEMT-1-18 was excised from the vector using Pst I and
20   Sac II, isolated from an agarose gel and labeled with ^{32}P by random primed synthesis using the Ready-To-GoÔ DNA labeling kit (cat#27-9251-01)from Pharmacia. This probe was used to screen a rat brain stem-spinal cord cDNA library in l ZAP II (Stratagene, cat# 936521). The filters were incubated with the probe for 18 hours at 65°C in 2x SSC, 5x Denhardt's solution and 0.2% SDS. The filters were rinsed twice in 0.1x SSC, 0.2% SDS at room temperature.
25   The filters were then washed twice for 45 min in 0.1x SSC, 0.2% SDS at 65°C, once for 45 min at 65°C in 5 mM EDTA, 0.2% SDS, pH 8.0 and finally rinsed with 0.1x SSC at room temperature.

Hybridization-positive phages were purified and their inserts rescued by helper phage mediated excision to yield plasmid DNA. The insert of plasmid pBS/1-18 was sequenced
30   progressively with the 1-18-specific primers. To generate a mammalian expression vector, a 2 Kb Sma I - Xho I fragment from pBS/1-18 was isolated and subcloned into the Eco RV and

Xho I sites of pcDNA3 (InVitrogen, San Diego, Ca). This expression vector was called pcDNA3-1-18. Plasmid DNA was prepared using the Qiaprep system from Qiagen.

Expression

HEK-293 cells were transfected with a mammalian expression construct coding for the 1-18 clone (pcDNA 3.0 vector, Invitrogen) using the Superfect reagent (Qiagen). A stable receptor pool of 1-18 was developed by applying a selection marker (G418, 0.6 mg/ml) and the cells were maintained in this selection medium. The presence of mRNA specific for clone 1-18 was assessed by Northern blot analysis and by the reverse transcriptase polymerase chain reaction (RT-PCR).

Ligands

In order to identify the ligand of clone 1-18, a collection of peptide and non-peptide ligands was obtained from commercial sources (Sigma, CalBiochem, American Peptide Company, Bachem, RBI). The compounds were dissolved in water/DMSO at 30 iM and placed in 96 well microplates. A total of 846 compounds (peptides and non-peptides) were prepared and tested.

Assay

A functional assay was performed with FLIPR (Fluorescent Imaging Plate Reader, Molecular Devices) using the fluorescent calcium indicator Fluo-3 (Molecular Probes) on a 96 well platform. HEK-293 cells, either expressing the receptor or wild type cells, were loaded with Fluo-3 as follows. Stable HEK-293 clones expressing 1-18 or parental cells were plated at a density of 70,000 cells/well in a 96 well plate. On the day of the experiment, the 1-18 cells were loaded with fluorescent solution (Dulbecco's modified medium with 10% fetal bovine serum containing 4 ified medium with 10% fetal bovine serum containing 4 Probes) on a 96 well platform. HEK-293 cells, either expressing the receptor or wild type cells, were loaded with Fluo-3 as follows. Stable HEK-2 BSA (pH 7.4). The cells were analyzed using the FLIPR system to measure the mobilization of intracellular calcium in response to different compounds.

II.   Results

HEK-293 cells endogenously express some GPCRs such as bradykinin receptors which can be used as an internal control for assays. The background signal was established with all

of the compounds in the parental HEK-293 cells (non-transfected) using the FLIPR assay. HEK-293 cells expressing the clone 1-18 were stimulated with all compounds and calcium responses were compared with those in parental HEK-293 cells. Only one compound, melanin concentrating hormone (MCH), consistently elicited signals in the transformed cells but not the wild type cells. This indicates that MCH is interacting with the recombinantly expressed receptor. Confirmation of this conclusion was obtained by the observation of a dose-response relationship with MCH in the cells transfected with 1-18, but not in the non-transfected cells or in cells transfected with several other different receptors. Thus, it has been established that clone 1-18 is, in fact, a specific receptor for MCH and that this receptor can be used to screen compounds which either mimic the action of MCH (agonists) or antagonize the action of MCH (antagonists).

Screening assays can be performed using the FLIPR assay described above. Alternatively, MCH can be iodinated and used as a tracer in radioligand binding assays on whole cells or membranes. Other assays that can be used include the GTPaS assay, adenylate cyclase assays, assays measuring inositol phosphates, and reporter gene assays (*e.g.*, those utilizing luciferase, aqueorin, alkaline phosphatase, etc.).

All references cited herein are fully incorporated by reference. Having now fully described the invention, it will be understood by those of skill in the art that the invention may be performed within a wide and equivalent range of conditions, parameters and the like, without affecting the spirit or scope of the invention or any embodiment thereof.

CLAIMS

1.   A method of assaying a test compound for its ability to bind to the SLC-1 receptor, comprising:

a)   incubating a cell expressing the SLC-1 receptor gene with melanin concentrating hormone (MCH) and said test compound; and

b)   determining the extent to which the binding of said MCH is displaced by said test compound.

2.   The method of claim 1, wherein said cell expressing SLC-1 is a recombinant cell that has been transformed with a heterologous SLC-1 gene.

3.   The method of claim 1, wherein said assay is a radioligand assay and said MCH or said test compound is radioactively labeled.

4.   The method of claim 1, wherein said assay is an enzyme-linked immunosorbent assay (ELISA) and either said MCH or said test compound is joined to an enzyme.

5.   The method of any one of claims 1-4, further comprising determining whether said test compound significantly increases or decreases either the adenyl cyclase or intracellular calcium concentration of said cell.

6.   A method of determining if a test compound is an agonist, antagonist or inverse agonist of MCH, comprising:

a)   incubating a cell expressing SLC-1 with said test compound;

b)   determining the intracellular adenyl cyclase activity or intracellular concentration of calcium of said cell during the incubation of step a);

c)   comparing the results obtained in step b) with the results obtained when incubations are performed in the absence of said test compound; and

d)   concluding that said test compound is an agonist of MCH if the level of adenyl cyclase activity or intracellular calcium is significantly higher in the presence of said test compound than in its absence, or concluding that said test compound is an antagonist of MCH if the level of adenyl cyclase activity or intracellular calcium concentration is significantly lower in the presence of said test compound than in its absence.

7.   The method of claim 6, wherein said cell is a recombinant cell that has been transformed with a heterologous SLC-1 gene.

8.   The method of either claim 6 or claim 7, wherein said cell expressing SLC-1 and test compound are incubated in a medium further comprising MCH.

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INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: G01N 33/567, C12Q 1/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: G01N, C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, BIOSIS, CONFERENCE PAPERS INDEX (CPI).

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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P,X	Nature, Volume 400, No 6741, July 1999, Chambers J. et al, "Melanin-concentrating hormone is the cognate ligand for the orphan G-protein-coupled receptor SLC-1", page 261 - page 265, see abstract and method in page 264	1-8
P,X	FEBS Lett, Volume 457, No 3, Sept 1999, Bachner D. et al, "Identification of melanin concentratinghormone (MCH) as the natu ral ligand for the orphan somatostatin-like receptor 1 (SLC-1).", page 522 - page 524, abstract	1-8

| X | Further documents are listed in the continuation of Box C. | X | See patent family annex. |

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"&" document member of the same patent family

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27 Sept 2000	0 2 -10- 2000

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C (Continuation).   DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	Nature, Volume 400, No 6471, July 1999, Saito Y. et al, "Molecular characterization of the melanin-concentrating-hormone receptor" page 265 - page 269 --	1-8
X	FEBS Lett, Volume 398, No 2-3, December 1996, Kolakowski LF Jr. et al, "Characterization of a human gene related to genes encoding somatostatin receptors.", page 253 - page 258, abstract --	1-8
X	WO 9618651 A1 (SMITHKLINE BEECHAM CORPORATION), 20 June 1996 (20.06.96) --	1-8
A	US 5849708 A (ELFTHERIA MARATOS-FLIER), 15 December 1998 (15.12.98) --	1-8
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(54) Title: MELANIN CONCENTRATING HORMONE RECEPTOR

(57) Abstract: The invention provides a method of identifying an MCH receptor agonist or antagonist, by contacting an MCH receptor with one or more candidate compounds under conditions wherein the MCH receptor produces a predetermined signal in response to an MCH receptor agonist, and identifying a compound that alters production of the predetermined signal. The invention also provides a method of identifying an MCH receptor ligand, by contacting an MCH receptor with one or more candidate compounds under conditions that allow selective binding between the MCH receptor and an MCH receptor ligand, and identifying a compound that selectively binds to the MCH receptor. Also provided are methods of identifying an individual having or susceptible to an MCH receptor-associated condition, by detecting MCH receptor nucleic acid or polypeptide in a sample. The invention further provides signaling compositions, which contain a recombinantly expressed MCH receptor and a recombinantly expressed Gα subunit of a G protein, or which contain a recombinantly expressed MCH receptor, a G protein, and a calcium indicator.

MELANIN CONCENTRATING HORMONE RECEPTOR

BACKGROUND OF THE INVENTION

The present invention relates generally to the
field of medicine and, more specifically, to therapeutic
5   and diagnostic methods and compositions related to
melanin concentrating hormone receptor.

Obesity, or excess deposition of body fat,
represents a primary health concern in industrialized
nations. Obesity correlates with and may trigger the
10   onset of serious medical conditions, including
hypertension, diabetes, cardiovascular disease and
psychological maladjustments. Whereas diet, exercise and
appetite suppressants can produce modest results in the
reduction of body fat deposits, no consistently effective
15   or practical treatment has been found for controlling
obesity and its physiological and psychological
consequences.

Pathologically decreased body weight, or
cachexia, which commonly occurs in chronic diseases such
20   as cancer and AIDS, is also a serious health concern.
The weight loss characteristic of cachexia has been
associated with several contributing factors, including
food aversion due to altered sensitivity to taste and
smell, malfunction of the gastrointestinal tract,
25   insufficient nutrient intake, and metabolic disturbances.

Melanin-concentrating hormone, or MCH, is a
small, cyclic neuropeptide that plays an important role
in regulating body weight, metabolism, and feeding
behavior. MCH was first isolated from the pituitary
30   gland of salmon, where it functions to regulate scale
color. Intracerebral administration of MCH peptide in

mammals has been shown to produce a dose-dependent stimulation of food intake, whereas mice deficient in MCH exhibit decreased body weight due to reduced feeding behavior and an inappropriately increased metabolic rate.

5   Expression of MCH is increased in the *ob* mouse model of obesity as well as in normal animals following fasting. Thus, it is clear that MCH plays a critical role in regulating body weight, metabolism and appetite.

In mammals, the pattern of MCH expression in

10 the brain is also consistent with MCH playing a role in regulating complex behavior and in controlling the hypothalamic-pituitary-adrenal axis. Administration of MCH to rats has also been shown to regulate behavior, such as increasing female sexual receptivity, increasing

15 anxiety, and antagonizing the effect of α-melanocyte stimulating hormone (α-MSH) on aggression and exploratory behavior.

In view of the important role of MCH in regulating body weight, behavior, and general neural and

20 endocrine functions, it would be beneficial to develop compounds that mimic or antagonize MCH activity. These compounds could be used as therapeutics in conditions in which abnormal body weight, behavior, or neural and endocrine functions play a role. However, the cell

25 surface receptor that binds MCH, and the signal transduction pathway initiated by receptor binding, have not previously been identified. Therefore, it has not been possible to develop rapid and reliable methods of screening for therapeutic compounds that can be used to

30 regulate or alter MCH-mediated physiological or pathological functions.

Thus, there exists a need to identify the MCH receptor and to develop methods of screening for compounds that bind to MCH receptor or mimic or antagonize MCH activity. The present invention satisfies

5   this need and provides related advantages as well.

SUMMARY OF THE INVENTION

The invention provides a method of identifying an MCH receptor agonist or antagonist. The method consists of contacting an MCH receptor with one or more

10   candidate compounds under conditions wherein the MCH receptor produces a predetermined signal in response to an MCH receptor agonist. A candidate compound that alters production of the predetermined signal is identified. The compound is characterized as an MCH

15   receptor agonist or antagonist.

The invention also provides a method of identifying an MCH receptor ligand. The method consists of contacting an MCH receptor with one or more candidate compounds under conditions that allow selective binding

20   between the MCH receptor and an MCH receptor ligand. A compound that selectively binds the MCH receptor is identified. The compound is characterized as an MCH receptor ligand.

Also provided are methods of identifying an

25   individual having or susceptible to an MCH receptor-associated condition. In one embodiment, the method consists of detecting MCH receptor nucleic acid molecule in a sample from the individual. Abnormal structure or expression of the MCH receptor nucleic acid

30   molecule in the sample indicates that the individual has or is susceptible to an MCH receptor-associated

condition.   In another embodiment, the method consists of
detecting MCH receptor polypeptide in a sample from the
individual. Abnormal expression or activity of the MCH
receptor polypeptide in the sample indicates that the

5   individual has or is susceptible to an MCH
receptor-associated condition.   MCH receptor-associated
conditions include disorders of body weight, mood,
memory, learning, sleep, dopaminergic system function,
reproduction or growth.

10   The invention also provides signaling
compositions.   In one embodiment, the signaling
composition contains a recombinantly expressed MCH
receptor and a recombinantly expressed Gα subunit of a G
protein.   In another embodiment, the signaling

15   composition contains a recombinantly expressed MCH
receptor, a G protein, and a calcium indicator.

BRIEF DESCRIPTION OF THE DRAWINGS

        Figure 1 shows the nucleotide sequence (SEQ ID
NO:1) and deduced amino acid sequence (SEQ ID NO:2) of

20   human melanin-concentrating hormone receptor (GPR24 or
SLC-1) (Kolakowski et al., FEBS Letters 398:253-258
(1996), GenBank accession number U71092).

        Figure 2 shows the nucleotide sequence (SEQ ID
NO:3) and deduced amino acid sequence (SEQ ID NO:4) of

25   rat melanin concentrating hormone receptor (SLC-1)
(Lakaye et al., Bioc. Biophys. Acta 1401:216-220 (1998),
GenBank accession number AF008650).

        Figure 3 shows purification of SLC-1 endogenous
ligand from rat brain extracts. Figure 3A shows a C18

30   reverse-phase HPLC elution profile.   Figure 3B shows a

kinetic of the $[Ca^{2+}]_i$ changes evoked by fraction 57, with
or without trypsin treatment. Figure 3C shows final
purification of active peptide by Sephasil C8 SC2.1/10
using SMART system. The inset panel shows the peak

5   increments in $[Ca^{2+}]_i$ induced by designated HPLC
fractions.

Figure 4 shows the specificity of interaction
between MCH and SLC-1. Figure 4A shows the alignment of
rat/human MCH sequence with salmon MCH, somatostatin 14

10 and cortistatin 14. Figure 4B shows $[Ca^{2+}]_i$ changes in
CHO cells transfected with SLC-1 and Gαq/i3 induced by
MCH, salmon MCH, somatostatin-14 (SST-14), cortistatin-14
(CS-14), α-MSH, NEI, and MGOP-14. Figure 4C, left, shows
dose-response curves for changes in $[Ca^{2+}]_i$ induced by

15 SLC-1 alone or SLC-1 coexpressed with Gαq/i3. Figure 4C,
right, shows inhibition of forskolin-stimulated cAMP
accumulation in CHO cells transfected with SLC-1 alone.

Figure 5 shows the distribution of SLC-1 mRNA.
Figure 5A shows Northern blot analysis from indicated rat

20 tissues using an SLC-1 cDNA probe (top panel) and G3PDH
control probe (bottom panel). Figure 5B shows
localization of SLC-1 transcripts in rat brain sections
by *in situ* hybridization. Ctx : cortex; AON: anterior
olfactory nucleus; TT: taenia tecta; Tu: olfactory

25 tubercle; Acb: nucleus accumbens; Pir: piroform cortex;
Hpx: hippocampus; Th: thalamus; Hyp: hypothalamus; Amy:
amygdala; LC: locus coeruleus.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the
30 identification of the receptor for melanin concentrating
hormone (MCH) and its signal transduction pathway. The

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invention thus provides novel compositions and methods
that can be used to identify compounds that specifically
bind to or modulate signaling through the MCH receptor.
Such compounds can be used therapeutically to prevent or
5   ameliorate MCH receptor-associated conditions, including
disorders of body weight, behavior, memory, learning,
mood, sleep, or movement. The invention also provides
methods of identifying an individual having or
susceptible to an MCH receptor-associated condition.
10 Such knowledge allows optimal medical care for the
individual, including appropriate genetic counseling and
prophylactic and therapeutic intervention.

The invention provides a method of identifying
an MCH receptor agonist or antagonist. The method
15 consists of contacting an MCH receptor with a candidate
compound under conditions wherein the MCH receptor
produces a predetermined signal in response to an MCH
receptor agonist, and identifying a compound that alters
production of the predetermined signal. A compound that
20 alters production of the predetermined signal is
characterized as an MCH receptor agonist or antagonist.

As used herein, the term "MCH receptor" refers
to a heptahelical membrane-spanning G-protein coupled
polypeptide, previously designated SLC-1 or GPR24, which,
25 as disclosed herein, is the endogenous receptor for
melanin-concentrating hormone. The term "MCH receptor"
encompasses native MCH receptor polypeptides from all
vertebrate species including but not limited to human,
non-human primate, rat, mouse, rabbit, bovine, porcine,
30 ovine, canine, feline, avian, reptile, amphibian or fish.
The human MCH receptor nucleotide sequence (SEQ ID NO:1)
and encoded amino acid sequence (SEQ ID NO:2) are
described in Kolakowski et al., FEBS Letters 398:253-258

(1996), and are shown in Figure 1. The rat MCH receptor
nucleotide sequence (SEQ ID NO:3) and encoded amino acid
sequence (SEQ ID NO:4) are described in Lakaye et al.,
Bioc. Biophys. Acta 1401:216-220 (1998), and are shown in
5   Figure 2. Based on the high degree of identity between
rat and human MCH receptor nucleotide and amino acid
sequences, it is predicted that MCH receptor from other
species will be highly homologous to the rat and human
MCH receptor.

10   The term "MCH receptor" also encompasses
polypeptides containing minor modifications with respect
to a native MCH receptor sequence, and fragments of full-
length MCH receptor, so long as the modified polypeptide
or fragment retains one or more of the biological
15   activities of a native MCH receptor, such as the ability
to selectively bind MCH, or the ability to couple to and
signal through a G protein in response to an MCH receptor
ligand. A modified polypeptide can have, for example,
one or more additions, deletions, or substitutions of
20   natural or non-natural amino acids relative to the native
polypeptide, so long as a biological activity of a native
MCH receptor is retained.

          Furthermore, the term "MCH .receptor"
encompasses MCH receptor polypeptides as they are found
25   in vertebrate host cells or tissues which express MCH
receptor, including but not limited to brain, eye,
skeletal muscle, tongue and pituitary, or as they are
present in membrane extracts or substantially pure
preparations derived from these tissues by standard
30   biochemical fractionation procedures. Additionally, the
term "MCH receptor" encompasses recombinantly expressed
MCH receptor polypeptides, modifications or fragments,
such as recombinant polypeptides expressed in cells or in

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cell lysates that support transcription and translation. Methods of producing recombinant polypeptides in cells and lysates are well known in the art. Likewise, the term "MCH receptor" includes chemically synthesized MCH
5   receptor polypeptides, which can be prepared by standard peptide synthesis methods.

The method of identifying an MCH receptor agonist or antagonist is practiced by contacting an MCH
10   receptor with a candidate compound under appropriate conditions in which MCH receptor produces a predetermined signal in response to a known MCH receptor agonist. As used herein, the term "candidate compound" refers to any molecule that potentially acts as an MCH receptor
15   agonist, antagonist or ligand in the screening methods disclosed herein. A candidate compound can be a naturally occurring macromolecule, such as a polypeptide, nucleic acid, carbohydrate, lipid, or any combination thereof. A candidate compound also can be a partially or
20   completely synthetic derivative, analog or mimetic of such a macromolecule or, a small organic molecule prepared by combinatorial chemistry methods. If desired in a particular assay format, a candidate compound can be detectably labeled or attached to a solid support.

25   Methods for producing pluralities of compounds, including chemical or biological molecules such as simple or complex organic molecules, metal-containing compounds, carbohydrates, peptides, proteins, peptidomimetics, glycoproteins, lipoproteins, nucleic acids, antibodies,
30   and the like, are well known in the art and are described, for example, in Huse et al., U.S. Patent No. 5,264,563; Francis et al., Curr. Opin. Chem. Biol. 2:422-428 (1998); Tietze et al., Curr. Biol., 2:363-371 (1998); Sofia, Mol. Divers. 3:75-94 (1998); Eichler et al., Med.

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<u>Res. Rev.</u> 15:481-496 (1995); and the like. Libraries
containing large numbers of natural and synthetic
compounds also can be obtained from commercial sources.

The number of different candidate compounds to
5   test in the methods of the invention will depend on the
application of the method. For example, one or a small
number of candidate compounds can be advantageous in
manual screening procedures, or when it is desired to
compare efficacy among several identified ligands,
10   agonists or antagonists. However, it is generally
understood that the larger the number of candidate
compounds, the greater the likelihood of identifying a
compound having the desired activity in a screening
assay. Additionally, large numbers of compounds can be
15   processed in high-throughput automated screening assays.
Therefore, "one or more candidate compounds" can contain,
for example, greater than about 10^3 different compounds,
preferably greater than about 10^5 different compounds,
more preferably, greater than about 10^7 different
20   compounds.

As used herein, the term "MCH receptor agonist"
refers to a compound that selectively promotes or
enhances normal signal transduction through the MCH
receptor. An MCH receptor agonist can act by any
25   agonistic mechanism, such as by binding an MCH receptor
at the normal MCH binding site, thereby promoting MCH
receptor signaling. An MCH receptor agonist can also
act, for example, by potentiating the binding activity of
MCH or signaling activity of MCH receptor. The methods
30   of the invention can advantageously be used to identify
an MCH receptor agonist that acts through any agonistic
mechanism.

10

As described herein, an example of an MCH
receptor agonist is the 19 amino acid MCH cyclic peptide
from rat or human having the amino acid sequence shown in
Figure 4A (SEQ ID NO:5). A further example of an MCH

5   receptor agonist is the 17 amino acid MCH cyclic peptide
from salmon shown in Figure 4A (SEQ ID NO:6). In
contrast, somatostatin-14 (Figure 4A, SEQ ID NO:7), the
somatostatin analog RC-160, cortistatin-14 (Figure 4A,
SEQ ID NO:8), cortistatin-29, MCH-precursor-derived

10   peptide NEI, MCH-gene-overprinted-polypeptide, MGOP-14,
MGOP-27, and α-melanotropin (MSH), as disclosed herein,
are not MCH receptor agonists, as they do not promote
signaling through the MCH receptor under conditions in
which MCH receptor produces a predetermined signal in

15   response to an MCH receptor agonist.

In contrast, as used herein, the term "MCH
receptor antagonist" refers to a compound that
selectively inhibits or decreases normal signal
transduction through the MCH receptor. An MCH receptor

20   antagonist can act by any antagonistic mechanism, such as
by binding an MCH receptor or MCH, thereby inhibiting
binding between MCH and MCH receptor. An MCH receptor
antagonist can also act, for example, by inhibiting the
binding activity of MCH or signaling activity of MCH

25   receptor. For example, an MCH receptor antagonist can
act by altering the state of phosphorylation or
glycosylation of MCH receptor. The methods of the
invention can advantageously be used to identify an MCH
receptor antagonist that acts through any antagonistic

30   mechanism.

An example of an MCH receptor antagonist is a
peptide or peptidomimetic derived from a portion of MCH
receptor that binds MCH. In rat MCH receptor, the ligand

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binding pocket is predicted to include residues Tyr230, of the fourth transmembrane domain, Phe266, of the fifth transmembrane domain, and Trp318, Tyr322 and Gln325, positioned in the sixth transmembrane domain (Kolakowski
5   et al., FEBS Letters 398:253-258 (1996)). Thus, a peptide or peptidomimetic that includes an MCH receptor amino acid sequence spanning one or more of these residues that constitute the binding pocket of MCH receptor can act as an MCH receptor antagonist.

10   Suitable assay conditions under which MCH receptor produces a predetermined signal in response to an MCH receptor agonist can be determined by those skilled in the art, and will depend on the particular predetermined signal one intends to detect. As used
15 herein, the term "predetermined signal" refers to a readout, detectable by any analytical means, that is a qualitative or quantitative indication of activation of signal transduction through the MCH receptor. As disclosed herein, MCH receptor couples to G proteins in
20 response to the MCH receptor agonist MCH. Therefore, any known or predicted G protein-coupled cellular event, such as elicitation of second messengers, induction of gene expression or altered cell proliferation, differentiation or viability, can be a "predetermined signal" that is an
25 indication of activation of signal transduction through the MCH receptor.

        As used herein the term "G protein" refers to a class of heterotrimeric GTP binding proteins, with subunits designated $G\alpha$, $G\beta$ and $G\gamma$, that couple to seven-
30 transmembrane cell surface receptors to transduce a variety of extracellular stimuli, including light, neurotransmitters, hormones and odorants to various intracellular effector proteins. The term "G protein"

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encompasses endogenous and recombinantly expressed G
proteins from all eukaryotic and prokaryotic organisms,
including mammals, other vertebrate organisms, Drosophila
and yeast. Also encompassed within the term "G protein"
5   are modifications and fragments of native G proteins that
maintain MCH receptor binding activity, signal
transduction activity, or both, of a native G protein.

        Four major classes of G proteins have been
identified, which are defined by their Gα subunits, Gαi,
10 Gαs, Gαq and Gα12. As disclosed herein, MCH receptor
couples to G proteins containing either Gαi and Gαq
subunits, and potentially couples to G proteins
containing other Gα subunits. Signaling through Gαi-
containing G proteins inhibits adenylyl cyclase activity,
15 which can be determined, for example, in an assay that
measures increased or decreased forskolin-stimulated cAMP
accumulation as the predetermined signal (see Example
III, below). Signaling through Gαq-containing G proteins
promotes calcium ion influx, which can be determined, for
20 example, in an assay that measures an increase or
decrease in intracellular Ca^{2+} as the predetermined signal
(see Examples I-III, below).

        The specificity of Gα subunits for cell-surface
receptors is determined by the C-terminal five amino
25 acids of the Gα. Thus, any convenient G-protein mediated
signal transduction pathway can be assayed by
constructing a chimeric Gα containing the C-terminal
residues of a Gα known or predicted to couple to MCH
receptor, with the remainder of the protein corresponding
30 to a Gα that couples to the signal transduction pathway
it is desired to assay. As used herein, the term
"chimeric Gα" refers to any functional Gα polypeptide
that contains at least the five C-terminal amino acids of

one Gα, with the remainder of the polypeptide including
amino acid sequences corresponding to one or more
different Gα subunits.

5   The nucleotide sequences and signal
transduction pathways of different classes and subclasses
of Gα subunits in a variety of eukaryotic and prokaryotic
organisms are well known in the art. Thus, one skilled
in the art can readily construct any desired chimeric Gα
10   by methods known in the art and described, for example,
in Conklin et al., Nature 363:274-276 (1993), and
Komatsuzaki et al., FEBS Letters 406:165-170 (1995). For
example, as described in Example I, below, a chimeric Gα
that contains amino acids 1-354 of a Gαq and the C-
15   terminal 5 residues of a Gαi3 can be constructed by PCR,
and used to couple MCH receptor to signaling through the
Gαq pathway. Likewise, a chimeric Gα useful in the
methods of the invention can include the C-terminal 5
residues of a Gαi and the N terminal residues of a
20   different Gαi, a Gαs or a Gα12. As MCH receptor also
interacts with Gαq (see Example III, below), a chimeric
Gα useful in the methods of the invention can
alternatively include, for example, the C-terminal 5
residues of a Gαq and the N terminal residues of a Gαi, a
25   Gαs or a Gα12.

           Signaling through G proteins containing various
Gα subunits can lead to increased or decreased production
or liberation of second messengers, including, for
example, arachidonic acid, acetylcholine, diacylglycerol,
30   cGMP, cAMP, inositol phosphate and ions; altered cell
membrane potential; GTP hydrolysis; influx or efflux of
amino acids; increased or decreased phosphorylation of
intracellular proteins; or activation of transcription.
Those skilled in the art can determine an appropriate

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assay for detecting alterations in any desired signal
transduction pathway in response to a candidate compound.
Exemplary assays, including high throughput automated
screening assays, to identify alterations in signal

5   transduction pathways and gene expression are described,
for example, in Gonzalez et al., Curr. Opin. in Biotech.
9:624-631 (1998) and in Jayawickreme et al., Curr. Opin.
Biotech. 8:629-634 (1997), and in references reviewed
therein. Yeast cell-based bioassays for high-throughput

10   screening of drug targets for G protein coupled receptors
are described, for example, in Pausch, Trends in Biotech.
15:487-494 (1997). A variety of cell-based expression
systems, including bacterial, yeast, baculovirus/insect
systems and mammalian cells, useful for detecting G

15   protein coupled receptor agonists and antagonists are
described, for example, in Tate et al., Trends in
Biotech. 14:426-430 (1996).

Assays to detect and measure signal
transduction can involve first contacting the cell,

20   extract or artificial assay system with a detectable
indicator. Calcium indicators, pH indicators, and metal
ion indicators, and assays for using these indicators to
detect and measure selected signal transduction pathways
are described, for example, in Haugland, Molecular Probes

25   Handbook of Fluorescent Probes and Research Chemicals,
Sets 20-23 and 25 (1992-94). Assays to determine changes
in gene expression can involve transducing cells with a
promoter-reporter nucleic acid construct such that, for
example, β-lactamase, luciferase, green fluorescent

30   protein or β-galactosidase will be expressed in response
to contacting MCH receptor with an agonist or antagonist.
Such assays and reporter systems are well known in the
art and are described, for example, at

http://www.aurorabio.com/tech_platform-
assay_technologies.html.

An assay to determine whether a candidate
compound is an MCH receptor agonist or antagonist can be
5   performed either in the presence or absence of a known
MCH receptor agonist, such as MCH. Thus, compounds that
directly promote or inhibit signaling through MCH
receptor, as well as compounds that indirectly affect the
normal interaction between MCH receptor and an agonist,
10 or the activity of MCH receptor or an agonist, can be
identified by the methods disclosed herein.

The invention also provides compositions useful
for identifying MCH receptor agonists and antagonists.
In one embodiment, the invention provides a signaling
15 composition containing a recombinantly expressed MCH
receptor and a recombinantly expressed Gα subunit of a G
protein. An example of such a composition is the CHO
cell line or HEK 293-T cell line expressing recombinant
MCH receptor and recombinant Gαq/i3, described in Example
20 II, below. As used herein, the term "signaling
composition" refers to any composition in which
contacting MCH receptor with an MCH receptor agonist will
elicit a predetermined signal.

In another embodiment, the invention provides a
25 signaling composition containing a recombinantly
expressed MCH receptor, a G protein, and a calcium
indicator. Calcium indicators and their use are well
known in the art, and include compounds like Fluo-3 AM,
Fura-2, Indo-1, FURA RED, CALCIUM GREEN, CALCIUM ORANGE,
30 CALCIUM CRIMSON, BTC, OREGON GREEN BAPTA, which are
available from Molecular Probes, Inc., Eugene Oreg., and
described, for example, in U.S. Patent Nos. 5,453,517,

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5,501,980 and 4,849,362. An example of a signaling
composition containing a recombinantly expressed MCH
receptor, endogenous G protein, and a calcium indicator
is the CHO cell line expressing recombinant MCH receptor
5   loaded with the calcium indicator Fluo-3 AM, described in
Example III, below.

      If desired, the Gα subunit of the G protein in
the signaling composition containing a recombinantly
expressed MCH receptor, a G protein, and a calcium
10   indicator can be recombinantly expressed. An example of
such a composition is the CHO cell line expressing
recombinant MCH receptor, recombinant Gαq/i3, and loaded
with the calcium indicator Fluo-3 AM, described in
Example I, below.

15   As used herein, the term "recombinantly
expressed," in reference to an MCH receptor or Gα subunit
of a G protein, refers to a polypeptide that is
transiently or stably expressed from a non-natural
nucleic acid molecule. Recombinant expression is
20   advantageous in providing a higher level of expression of
the polypeptide than is found endogenously, and also
allows expression in cells or systems in which the
polypeptide is not normally found. A "non-natural"
nucleic acid molecule is one that has been constructed,
25   at least in part, by molecular biological methods, such
as PCR, restriction digestion and ligation. A non-
natural nucleic acid expression construct generally will
contain a constitutive or inducible promoter of RNA
transcription appropriate for the host cell or
30   transcription-translation system, operatively linked to a
nucleotide sequence that encodes the polypeptide of
interest. The expression construct can be DNA or RNA,
and optionally can be contained in a vector, such as a

plasmid or viral vector. Given knowledge of the nucleic
acid sequence encoding MCH receptor and various Gα
subunits of G proteins, one skilled in the art can
recombinantly express these polypeptides using routine
5   laboratory methods, described, for example, in standard
molecular biology technical manuals, such as Sambrook et
al., Molecular Cloning: A Laboratory Manual, Cold Spring
Harbor Laboratory, New York (1992) and Ansubel et al.,
Current Protocols in Molecular Biology, John Wiley and
10   Sons, Baltimore, MD (1989).

        The signaling compositions of the invention
include, for example, cells, cell extracts and
reconstituted artificial signaling systems. The cell
compositions of the invention include any cell type in
15   which MCH receptor can couple to a G protein and induce a
detectable signal in response to an agonist, such as a
vertebrate cell, insect cell (e.g. Drosophila), yeast
cell (e.g. S. cerevisiae, S. pombe, or Pichia pastoris)
or prokaryotic cell (e.g. E. coli). Exemplary vertebrate
20   cells include, but are not limited to, mammalian primary
cells and established cell lines, such as COS, CHO, HeLa,
NIH3T3, HEK 293-T, PC12, and amphibian cells, such as
Xenopus embryos and oocytes. Also included are cells
from transgenic animals, such as transgenic mice, that
25   have been engineered by known methods to express
recombinant MCH receptor or Gα subunit.

        The signaling compositions of the invention
also include crude or partially purified lysates or
extracts of the cell compositions of the invention, and
30   reconstituted artificial signaling systems. Artificial
signaling systems can include, for example, a natural or
artificial lipid bilayer, such as a liposome, to maintain
MCH receptor in a natural configuration, and cellular

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fractions or isolated components necessary for
transducing and detecting the desired predetermined
signal.

The invention also provides a method of
5   identifying an MCH receptor ligand. The method consists
of contacting an MCH receptor with one or more candidate
compounds under conditions that allow selective binding
between MCH receptor and an MCH receptor ligand. A
compound that selectively binds MCH receptor is
10 identified, and the compound is characterized as an MCH
receptor ligand.

As used herein, the term "MCH receptor ligand"
refers to any biological or chemical compound that
selectively binds an MCH receptor polypeptide. An "MCH
15 receptor ligand" can be an agonist or antagonist of MCH
receptor, as described above, or can be a compound having
little or no effect on MCH receptor signaling, so long as
the compound selectively binds an MCH receptor
polypeptide. An MCH receptor ligand can be used to
20 specifically target a diagnostic or therapeutic moiety to
a region of the brain, or other organ or tissue of the
body, that expresses MCH receptor. Thus, an MCH receptor
ligand can be labeled with a detectable moiety, such as a
radiolabel, fluorochrome, ferromagnetic substance, or
25 luminescent substance, and used to detect expression of
MCH receptor polypeptide in an isolated sample or in *in
vivo* diagnostic imaging procedures. Likewise, an MCH
receptor ligand can be labeled with a therapeutic moiety,
such as a cytotoxic or cytostatic agent or radioisotope,
30 and administered in an effective amount to arrest
proliferation or kill a cell or tissue that expresses MCH
receptor. Thus, an MCH receptor ligand labeled with a
therapeutic moiety can be used to treat proliferative

diseases, including cancer and inflammatory diseases,
that affect MCH receptor-expressing tissues, or as an
alternative to neurosurgery to ablate regions of the
brain responsible for MCH receptor-associated conditions,
5   such as the conditions described below.

An MCH receptor ligand that selectively binds
MCH receptor will bind MCH receptor with high affinity,
but will not bind, or bind with low affinity, to a
structurally related receptor that is not an MCH
10   receptor, such as a somatostatin receptor. High affinity
binding refers to a dissociation constant (Kd) of less
than about 10^{-6} M, preferably less than about 10^{-7} M, such
as less than about 10^{-8} M. In contrast, low affinity
binding refers to a Kd of about 10^{-4} M or more.

15   An example of an MCH receptor ligand is
mammalian or salmon MCH which, as disclosed herein, binds
and activates MCH receptor with a half-maximal response
at nanomolar concentration. A further example of an MCH
receptor ligand is an antibody specific for MCH receptor,
20   such as an antibody specific for an extracellular region
of an MCH receptor. In order to prepare an antibody
specific for an extracellular region of MCH receptor, a
peptide containing substantially the sequence of one of
the three extracellular loops of MCH receptor, such as
25   substantially the sequence HQLMGNGVWHFGETMCT (SEQ ID
NO:9), RLIPFPGGAVGCGIRLPNPDTDL (SEQ ID NO:10),
QLISISRPTLTFVY (SEQ ID NO:11), or immunogenic fragment
therefrom, or substantially the N-terminal sequence
MLCPSKTDGSGHSGRIHQETHGEGKRDKISNSEGRENGGRGFQMNGGSLEAEHASRM
30   SVLRAKPMSNSQRLLLLSP (SEQ ID NO:12), or immunogenic
fragment therefrom, can be produced, either by direct
synthesis, by recombinant means, or by enzymatic
digestion of MCH receptor. The peptide can formulated in

20

an immunogenic composition, such as conjugated to a carrier protein or formulated with an adjuvant, to generate an MCH receptor specific polyclonal or monoclonal antibody using methods well known in the art

5   and described, for example, in Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York (1989). Methods of preparing fragments of antibodies with specific binding activity, such as Fab fragments, and methods of preparing

10 recombinant, chimeric or humanized antibodies directed against a peptide sequence, are also well known in the art, and such antibodies and fragments directed against MCH receptor are also contemplated as MCH receptor ligands.

15

       A variety of low- and high-throughput assays suitable for detecting selective binding interactions between a receptor and a ligand are known in the art. Both direct and competitive assays can be performed,

20 including, for example, fluorescence correlation spectroscopy (FCS) and scintillation proximity assays (SPA) reviewed in Major, J. Receptor and Signal Transduction Res. 15:595-607 (1995); and in Sterrer et al., J. Receptor and Signal Transduction Res. 17:511-520

25 (1997)). Other assays for detecting binding interactions include, for example, ELISA assays, FACS analysis, and affinity separation methods, which are described, for example, in Harlow and Lane, Eds., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory (1988).

30 Such assays can be performed, for example, with whole cells that express MCH receptor, membrane fractions therefrom or artificial systems, as described previously, or with substantially purified MCH receptor polypeptide, either in solution or bound to a solid support.

21

The MCH receptor ligands, agonists and antagonists identified using the methods and compositions of the invention can be isolated and administered to an individual, such as a human or other mammal, in an
5 effective amount to prevent or ameliorate the severity of an MCH receptor-associated condition. As used herein, the term "MCH receptor-associated condition" refers to any pathological condition associated with a tissue or cell in which MCH receptor is expressed. In particular,
10 the term "MCH receptor-associated condition" includes any abnormal physiological or psychological condition in which a quantitative or qualitative alteration in signaling through the MCH receptor contributes to the symptoms of the condition. An MCH receptor-associated
15 condition also includes any physiological or psychological condition in which altering signaling through the MCH receptor has a beneficial effect in the individual.

20   An MCH receptor-associated condition can have any of a variety of causes, including genetic, environmental and pathological causes. For example, an MCH receptor-associated condition can be caused by a mutation in MCH receptor nucleic acid that alters its
25 expression or structure, a mutation in MCH, or a mutation in a molecule that normally regulates expression or bioavailability of MCH or MCH receptor. An MCH receptor-associated condition can also be caused by environmental factors, such as exposure to toxins, therapeutic drugs or
30 hormones that alter signaling through the MCH receptor, or affect the viability or function of cells or tissues that express the MCH receptor, MCH agonists and antagonists, or their regulatory molecules. An MCH receptor-associated condition can also be due to a
35 pathological condition that affects the viability or

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function of cells or tissues that express the MCH
receptor, MCH agonists and antagonists, or their
regulatory molecules, such as neurodegenerative diseases,
infectious diseases, endocrine disorders, and benign and
5 malignant tumors and tumor metastases.

As disclosed herein, MCH receptor is expressed
in regions of the brain involved in taste, olfaction,
feeding behavior and metabolism. Furthermore, central
administration of MCH promotes feeding (Ludwig et al.,
10 Am. J. Physiol. 274:E627-E633 (1998)), and MCH mRNA
amounts rise as a result of starvation and leptin
deficiency (Qu et al., Nature 380:243-247 (1996)). In
contrast, MCH deficiency results in reduced body weight
and leanness due to reduced feeding and inappropriately
15 increased metabolic rate (Shimada et al., Nature 396:670-
674 (1998)). Therefore, MCH receptor-associated
conditions can include disorders of body weight and
metabolism, including disorders involving increased body
weight, such as moderate or severe obesity due to
20 endocrine dysfunction or overfeeding, and disorders
involving decreased body weight, such as moderate
underweight or cachexia. The term "cachexia" refers to a
general weight loss and wasting occurring in the course
of a chronic disease, such as cancer or AIDS, or as a
25 result of emotional disturbance, such as anorexia. Thus,
an MCH receptor agonist, antagonist or ligand can be used
as a drug to restore more normal weight, metabolism and
feeding behavior.

As also disclosed herein, MCH receptor is
30 expressed in regions of the brain involved in
dopaminergic-modulated responses. Therefore, MCH
receptor-associated conditions include pathologies
associated with dopamine insufficiency or excess,

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including, but not limited to, Parkinson's disease and parkinsonian syndromes, Huntington's disease, and drug- and toxin-induced movement disorders caused by altered availability or activity of dopamine. Thus, MCH receptor

5 agonists and antagonists can be used as therapeutics to prevent or treat conditions due to altered dopaminergic system function.

As further disclosed herein, MCH receptor is expressed in regions of the brain involved in control of

10 behavior, memory and learning, mood and sleep. Disorders of behavior include, but are not limited to, autistic disorder, Asperger's disorder, aggression, pervasive developmental disorders, Tourette's syndrome, attention- deficit hyperactivity disorder and addiction. Disorders

15 of memory and learning include, but are not limited to, Alzheimer's disease; dementia, including dementia due to neurodegenerative diseases, infectious disease, proliferative diseases, endocrine disease, tumors, metabolic disorders, and toxins; and developmental

20 learning disabilities. Disorders of sleep and of the sleep-wake cycle include, but are not limited to, insomnia, bedwetting, sleepwalking, sleep apnea and narcolepsy. Disorders of mood include, but are not limited to, depression; anxiety disorders, such as

25 generalized anxiety disorder, panic attacks, obsessive- compulsive disorder, phobias, acute stress disorder, post-traumatic stress disorder; and psychotic disorders, such as unipolar mania or depression, bipolar disorder and schizophrenia. Thus, MCH receptor agonists,

30 antagonists and ligands can be used as therapeutics to prevent or treat disorders of behavior, memory and learning, mood and sleep.

24

As further disclosed herein, MCH receptor is expressed in the pituitary, which controls various reproductive functions and developmental growth. Thus, an MCH receptor agonist or antagonist can be used as a
5 male or female contraceptive, or in treatment of an MCH receptor-associated reproductive disorder, such as male and female sexual dysfunction, impotence, failure of lactation, infertility and precocious puberty, or an MCH receptor-associated growth disorder, such as dwarfism or
10 acromegaly.

The MCH receptor agonist, antagonist or ligand therapeutics of the present invention can be conveniently formulated for administration together with a pharmaceutically acceptable carrier. Suitable
15 pharmaceutical carriers for the methods of the invention are well known and include, for example, aqueous solutions such as physiologically buffered saline, and other solvents or vehicles such as glycols, glycerol, oils or injectable organic esters. A pharmaceutical
20 carrier can contain a physiologically acceptable compound that acts, for example, to stabilize or increase the solubility of a pharmaceutical composition. Such a physiologically acceptable compound can be, for example, a carbohydrate, such as glucose, sucrose or dextrans; an
25 antioxidant, such as ascorbic acid or glutathione; a chelating agent; a low molecular weight protein; or another stabilizer or excipient. Pharmaceutical carriers, including stabilizers and preservatives, are described, for example, in Martin, Remington's Pharm.
30 Sci., 15th Ed. (Mack Publ. Co., Easton, 1975).

Those skilled in the art can formulate the therapeutic compunds to ensure proper distribution in vivo. For example, the blood-brain barrier (BBB)

25

excludes many highly hydrophilic compounds. To ensure
that the therapeutic compounds of the invention cross the
BBB, they can be formulated, for example, in liposomes,
or chemically derivatized. Those skilled in the art
5   understand that the choice of the pharmaceutical
formulation and the appropriate preparation of the
composition will depend on the intended use and mode of
administration.

     Methods of introduction of a therapeutic
10   compound of the invention include, but are not limited
to, intradermal, intramuscular, intraperitoneal,
intravenous, subcutaneous, oral, intranasal, intraspinal
and intracerebral routes. Methods of introduction can
also be provided by rechargable or biodegradable devices,
15   particularly where gradients of concentrations of drug in
a tissue is desired. Various slow release polymeric
devices are known in the art for the controlled delivery
of drugs, and include both biodegradable and non-
degradable polymers and hydrogels.

20   An effective dose of a therapeutic composition
of the invention can be determined by extrapolation from
the concentration required for modulating MCH receptor
signaling or binding in *in vitro* assays described herein,
and from the dose required for efficacy in an animal
25   model of the MCH receptor-associated conditions described
herein. Typically, an appropriate dose can be in the
range of 0.001-100 mg/kg of body weight, but can be
determined by those skilled in the art depending on the
bioactivity of the particular compound, the desired route
30   of administration, the gender and health of the
individual, the number of doses and duration of
treatment, and the particular condition being treated.

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The invention also provides methods of identifying an individual having or susceptible to an MCH receptor-associated condition. Such knowledge allows optimal medical care for the individual, including
5   appropriate genetic counseling and prophylactic and therapeutic intervention.

In one embodiment, the method consists of detecting MCH receptor nucleic acid molecule in a sample from the individual. Abnormal structure or expression of
10   MCH receptor nucleic acid molecule in the sample indicates that the individual has, or is at greater risk than a normal individual of developing, an MCH receptor-associated condition.

As used herein, the term "MCH receptor nucleic
15   acid molecule" refers to a DNA or RNA molecule that corresponds to at least a part of a nucleotide sequence of a gene that encodes an MCH receptor. For example, an MCH receptor nucleic acid molecule can be MCH receptor genomic DNA, mRNA, or a nucleic acid molecule derived
20   therefrom, such as a PCR amplification product or cDNA. An MCH receptor nucleic acid molecule can correspond to the sense or antisense strand, and can include coding or non-coding sequence, or both, of an MCH receptor gene. Normal human MCH receptor cDNA has substantially the
25   nucleotide sequence presented in Figure 1 (SEQ ID NO:1), and encodes substantially the amino acid sequence presented in Figure 1 (SEQ ID NO:2).

By detecting MCH nucleic acid in a sample,
30   either altered expression or structure of the nucleic acid molecule can be determined, and used to diagnose or predict risk of developing an MCH receptor-associated condition. As used herein, the term "altered expression"

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of an MCH receptor nucleic acid molecule refers to an
increased or decreased amount of MCH receptor nucleic
acid in the test sample relative to levels in a normal
sample.   Altered abundance of a nucleic acid molecule can

5   result, for example, from an altered rate of
transcription, from altered transcript stability, or from
altered copy number of the corresponding gene. As used
herein, the term "altered structure" of a nucleic acid
molecule refers to differences, such as point mutations,

10   insertions, deletions, chromosomal translocations, splice
variations and other rearrangements, between the
structure of a nucleic acid molecule of the invention in
a test sample and the structure of the nucleic acid
molecule in a normal sample. Those skilled in the art

15   understand that mutations that alter the structure of a
nucleic acid molecule can also alter its expression.
Abundance or structure of MCH receptor in a normal sample
can, if desired, be determined simultaneously with the
test sample, or can be a previously established value.

20   As used herein, the term "sample" refers to any
biological fluid, cell, tissue, organ or portion thereof,
that is appropriate to detect MCH receptor nucleic acids
and polypeptides, and includes samples present in an
individual as well as samples obtained or derived from

25   the individual. For example, a sample can be a
histologic section of a specimen obtained by biopsy, or
cells that are placed in or adapted to tissue culture. A
sample further can be a subcellular fraction or extract,
or a crude or substantially pure nucleic acid or protein

30   preparation.

         The appropriate source and method of preparing
the sample can be determined by those skilled in the art,
depending on the application of the detection method.

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For example, in order to detect structure of MCH receptor
genomic DNA, any convenient source of DNA, such as blood
cells, lymph cells, cheek cells or skin cells, can be
used. However, to detect expression of MCH receptor mRNA
5   or protein, or determine receptor activity, a sample
should be obtained from a tissue that expresses MCH
receptor, such as neural tissue or, more conveniently,
tongue or skeletal muscle.

Various qualitative and quantitative assays to
10 detect altered expression or structure of a nucleic acid
molecule in a sample are well known in the art, and
generally involve hybridization of a detectable agent,
such as a complementary primer or probe, to the nucleic
acid molecule. Such assays include, for example, *in situ*
15 hybridization, which can be used to detect altered
chromosomal location of the nucleic acid molecule,
altered gene copy number, or altered RNA abundance,
depending on the format used. Other assays include, for
example, Northern blots and RNase protection assays,
20 which can be used to determine the abundance and
integrity of RNA; Southern blots, which can be used to
determine the copy number and integrity of DNA; SSCP
analysis, which can detect single point mutations in DNA,
such as in a PCR or RT-PCR product; direct sequencing of
25 nucleic acid fragments, such as PCR amplification
fragments; and coupled PCR, transcription and translation
assays, such as the Protein Truncation Test, in which a
mutation in DNA is determined by an altered protein
product on an electrophoresis gel. An appropriate assay
30 format and detectable agent to detect an alteration in
the expression or structure of an MCH receptor nucleic
acid molecule can be determined depending on the
alteration it is desired to identify. Methods of
performing such assays are well known in the art.

The invention also provides a method of
identifying an individual having or susceptible to an MCH
receptor-associated condition, by detecting MCH receptor
polypeptide in a sample from the individual. Abnormal
5   structure or activity of MCH receptor polypeptide in the
sample indicates that the individual has or is
susceptible to an MCH receptor-associated condition.

As used herein, the term "altered expression"
of a polypeptide refers to an increased or decreased
10   amount, or altered subcellular localization, of the
polypeptide in the test sample relative to known levels
or localization in a normal sample. Altered abundance of
a polypeptide can result, for example, from an altered
rate of translation or altered copy number of the
15   corresponding message, or from altered stability of the
protein. Altered subcellular localization can result,
for example, from truncation or inactivation of a sorting
sequence, from fusion with another polypeptide sequence,
or altered interaction with other cellular polypeptides.

20   Various assays to detect altered expression of
polypeptides are known in the art, and generally involve
hybridization of a detectable agent, such as a labeled
ligand, to the polypeptide in a sample, or within the
body in diagnostic imaging procedures. Assays to detect
25   altered expression of MCH receptor can be performed *in
situ*, in which a detectably labeled ligand, such as an
antibody or other ligand identified by the methods
described herein, contacts MCH receptor in a whole cell.
Other assays to detect altered expression of MCH receptor
30   polypeptide include, for example, ELISA assays,
immunoprecipitation, and immunoblot analysis, which can
be performed with cell or tissue extracts. An
appropriate assay format and detectable agent to detect

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an alteration in the expression of MCH receptor
polypeptide can be determined depending by those skilled
in the art depending on the alteration it is desired to
identify. Methods of performing such assays are well

5   known in the art.

        Assays to determine activity of MCH receptor
have been described above in connection with screening
assays to identify MCH receptor agonists and antagonists,
and exemplary assays are described in Examples I-III,

10 below. Therefore, one skilled in the art can use such
assays to detect qualitatively or quantitatively altered
activity of MCH receptor in a sample, compared with
normal activity.

        The following examples are intended to

15 illustrate but not limit the present invention.

EXAMPLE I
MCH Receptor Assay System

        This example shows an assay system and
signaling composition that can be used to identify MCH

20 receptor agonists and antagonists. This assay system was
used to identify MCH as an endogenous agonist of MCH
receptor.

        SLC-1 exhibits about 40% amino acid identity to
five known human somatostatin recetors(SSTRs), which are

25 Gα-linked receptors. On the assumption that SLC-1 would
also bind a peptidic ligand and couple to Gαi proteins,
different tissues were harvested that were known to
express SLC-1, and processed for peptide extraction
following protocols described in Reinscheid et al.,

30 Science 270:792-794 (1995); Meunier et al., Nature

377:532-535 (1995); and Hinuma et al., Nature 393:272-276
(1998). Initially, chromatographic fractions, prepared
as described in Example II, were tested for their ability
to induce a decrease in cAMP levels in forskolin-
5   stimulated, SLC-1-transfected CHO cells. None of the
fractions showed a response which could be reproducibly
followed over several purification steps due to the
lability of the cAMP assays.

        A new assay system was therefore developed to
10 monitor SLC-1 reactivity through recording of calcium
influxes, by forcing SLC-1 to couple to a Gαq protein.
Because it has been shown that the five C-terminal
residues of Gα are sufficient for receptor contact, while
the rest of the subunit serves to interact with the
15 effector molecule, a Gαq/i3 chimera designed to drive
SLC-1 to Gαq activation was constructed. The Gαq/i3
chimera contained the five C-terminal residues of Gαi3
(ECGLY) while retaining the rest of the Gαq sequence,
residues 1-354.

20   Calcium influx assays were performed in CHO
cells transiently cotransfected with the Gαq/i3 chimera
and SLC-1. Construction of the Gαq/i3 chimera by PCR is
described in Conklin et al., Nature 363:274-276 (1993),
and Komatsuzaki et al., FEBS Lett. 406: 165-170 (1997).
25 The full-length rat SLC-1 cDNA was cloned by PCR using
specific oligonucleotides (described in Lakaye et al.,
Biochem. Biophys. Acta. 1401:216-220 (1997)) from a rat
brain Marathon cDNA library (Clontech). The resulting
1.1kb PCR products was subcloned into pcDNA 3.1 (+)
30 expression vector and sequenced. For transient
transfection, the SLC-1 cDNA subcloned into pcDNA 3.1 (+)
was transfected with the Gαq/i3 chimera into CHO dhfr (-)
cells using LipofectAMINE PLUS transfection reagent and

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following the manufacture's instructions (GIBCO-BRL).

Calcium influx assays were performed as
described in Coward et al., Proc. Natl. Acad. Sci. USA
5 95:352-357 (1998). In brief, transfected or control
cells were seeded into 96 wells at 5.5 x 10^4 cells/well.
The cells were loaded with Fluo-3 AM (Molecular Probes)
in standard bath solution (130mM NaCl, 2mM CaCl$_2$, 5mM KCl,
10mM glucose, 0.45mM KH$_2$PO$_4$, 0.4mM Na$_2$HPO$_4$, 8mM MgSO$_4$,
10 4.2mM NaHCO$_3$, 20mM HEPES, and 10 µM probenecid) with 0.1%
fetal bovine serum for 1 hr at 37°C, then washed with a
standard bath solution. Transient changes in [Ca^{2+}]$_i$
evoked by fractions were monitored by the FLIPR system
(Fluorometric Imaging Plate Reader, Molecular Devices) in
15 96 well plates at 488 nm for 210 seconds.

As described in Examples II and III below, MCH
receptor agonists induce dose-dependent transient
increases in cytoplasmic calcium levels in SLC-1-Gαq/i3
transfected cells using the above-described assay system.

20   **EXAMPLE II**

Identification of SLC-1 (GPR24) as MCH receptor

This example shows the purification of an
endogenous agonist of the orphan G-protein coupled
receptor SLC-1 (GPR24) and its identification as the
25 neuropeptide melanin-concentrating hormone (MCH).

The purification of the endogenous SLC-1 ligand
was performed as follows. 400g rat frozen brain (Pel-
Freez) were extracted in 1M acetic acid and centrifuged
30 at 20,000 x g for 15min at 4°C. The resulting supernatant
was precipitated with acetone and extracted with
diethylether. The aqueous phase was concentrated and

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loaded onto a C18 reverse phase HPLC column (PrepPAK-
Delta-Pac 25 x 100mm, Waters) and eluted with a linear
gradient of 5-48% CH$_3$CN in 0.1% trifluoroacetic acid (TFA)
at a flow rate of 1 ml/min. Fractions were monitored for
5   ability to induce increases in [Ca^{2+}]$_i$ in CHO cells
transiently cotransfected with the Gαq/i3 chimera and
SLC-1, using the calcium influx assay described in
Example I, above.

        Two consecutive HPLC fractions were identified
10 that elicited a robust increase of cytoplasmic calcium
levels in CHO cell cotransfected with the Gαq/i3 chimera
and SLC-1, as shown in Figures 3A and 3B. The activities
detected in these two fractions, 56 and 57, were specific
to the SLC-1-Gαq/i3 system, since they did not induce Ca^{2+}
15 influx in cells transfected with ORL-1-Gαq/i3 (Figure
3A). The same fractions also elicited an increase in
calcium levels upon cotransfection of SLC-1-Gαq/i3 into
HEK 293-T cells.

        The increase in calcium levels appeared to be
20 mediated by a peptide in the active fractions, since
trypsin treatment abolished activity (see Figure 3B,
dotted line). Trypsin treatment was performed by
incubating fraction 57 with 20 mU trypsin attached to
agaraose beads for 3h at 37°C. The reaction was
25 terminated by removing the beads by centrifugation.

        Active fractions 56 and 57 from the reverse
phase HPLC were pooled and further purified by six more
chromatographic steps. Briefly, pooled fractions 56 and
57 were further purified on a cation-exchange column AP-
30 1/SP-8HR (Waters) with a linear gradient of 0.15-0.35M
NaCl in 6mM HCl and 30% CH$_3$CN. Active fractions were
further purified on an analytical C18 Select B column

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(Merck) with a linear gradient of 21-33% CH_3CN in 0.1%
TFA. Positive fractions were then serially fractionated
in a SMART system on a Sephasil C8 SC2.1/10 column
(Pharmacia) with a linear gradient of 36-42% CH_3CN in 0.1%
5   TFA, a Sephasil C8 SC2.1/10 column (Pharmacia) with a
linear gradient of 33-48% CH3CN in 0.1%
heptafluorobutyric acid (HFBA), on a mRPC C2/C18 SC2.1/10
column (Pharmacia) with a linear gradient of 34.5-35.1%
CH_3CN in 0.1% TFA, and finally on a Sephasil C8 SC2.1/10
10 (Pharmacia) with a linear gradient of 26.4-27.6.% CH_3CN in
0.1% TFA at a flow rate of 0.1 ml per minute. The final
purification of the active compound by Sephasil C8
SC2.1/10 chromotography is shown in Figure 3C. The inset
panel represents the peak increments in $[Ca^{2+}]_i$ induced by
15 designated fractions. The solid line indicates
absorption at 214 nm and the dotted line indicates the
percentage of CH3CN.

The final active compound was subjected to a
structural analysis by MALDI mass spectrometry and Edman
20 degradation. Amino acid sequences were determined in a
pulse liquid automatic sequencer. Amino acid sequence
analysis revealed an N-terminal sequence in which three
of the first five residues were identical to that of the
rat melanin-concentrating hormone (MCH) described by
25 Vaughan et al., Endocrinology 125:1660-1665 (1989).
Synthetic rat MCH was shown to behave identically to the
purified active peptide in retention time (by reverse
phase HPLC) and in molecular size (by mass data).
Therefore, it was inferred that the isolated peptide was
30 MCH. Final yields of MCH was approximately 25pmol/kg of
rat brain (wet weight).

To confirm that the isolated peptide had the
same activity as MCH, synthetic rat MCH was assayed in

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CHO cells cotransfected with the Gαq/i3 chimera and SLC-
1. Synthetic rat MCH induced a dose-dependent transient
increase in $[Ca^{2+}]_i$ in transiently transfected rat SLC-1-
Gαq/i3 cells (see Figure 4B), but failed to induce

5   detectable $[Ca^{2+}]_i$ changes in mock transfected CHO cells
(data not shown). The MCH concentration required to
induce half-maximum response (EC50) was calculated to be
4.8 ± 0.5 nM, thus confirming that MCH is an endogenous
ligand for SLC-1.

10   MCH from other species were also tested in SLC-
1-Gαq/i3 transfected cells. Salmon MCH, described by
Kawauchi et al., Nature 305;321-323 (1983), which has a
high degree of homology to rat MCH in its central and C-
terminal portions, activated SLC-1 with an EC50 of 18.6 ±

15 2.3 nM. A longer isoform of human SLC-1, described by
Kolakowski et al., FEBS Lett. 398:255-258 (1996), was
inactive in this assay system.

        Because SLC-1 shares sequence similarities with
the somatostatin receptors and because somatostatin

20 exhibits a circular topology similar to that of MCH, as
shown in Figure 4A, somatostatin-14 and the somatostatin
analogue RC-160 were tested on SLC-1-Gαq/i3 transfected
cells. They were found to be inactive at inducing $[Ca^{2+}]_i$
increase in the cell assay (Figure 4B). Cortistatin-14

25 or -29, described in De Lecea et al., Nature 381:242-245
(1996), which are structurally related to somatostatin
(see Figure 4A), also failed to induce detectable $[Ca^{2+}]_i$
changes (see Figure 4B). Moreover, somatostatin-14 and
cortistatin-14 or -29 did not act as antagonists of the

30 MCH-activated SLC-1 response (not shown).

        Furthermore, MCH-gene related peptides (MCH-
precursor-derived peptide NEI, MCH-gene-overprinted-

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polypeptide, MGOP-14, or -27) (Nahonet al., <u>Endocrinology</u>
125:2056-2065 (1989); Toumaniantz et al., <u>Endocrinology</u>
137:4518-4521 (1996)), and α-melanotropin (MSH) also
failed to induce a transient increase in $[Ca^{2+}]_i$ in

5   transfected SLC-1-Gaq/i3 cells (see Figure 4B). Thus,
SLC-1 is a receptor specific for MCH, and consequently
the other peptides derived from the MCH gene, if
bioactive, must bind to different receptors.

MCH and α-MSH demonstrate opposite actions on

10   skin coloration in teleost fishes (Baker, <u>Ann. NY Acad</u>.
<u>Sci</u>. 680:279-289 (1993)) and exert antagonistic influence
on a variety of physiological function including feeding
behavior (Miller et al., <u>Peptides</u> 14:1-10 (1993);
Gonzalez et al., <u>Peptides</u> 17:171-177 (1996); Sanchez et

15   al., <u>Peptide</u> 18:393-396 (1997); and Ludwig et al., <u>Am. J.</u>
<u>Physiol</u>. 274:E627-E633. (1998)). It has also been
reported that MCH antagonizes the effect of NEI on
grooming and locomotor activities (Sanchez et al.,
<u>Peptide</u> 18:393-396 (1997)). When tested in the SLC-1-

20   Gaq/i3 transfected cell system at concentrations of 1 nM-
1 μM, neither α-MSH nor NEI was able to block the ability
of MCH to induce calcium mobilization (data not shown).
Since it is known that MCH is not recognized by the
melanocortin receptors (Ludwig et al., <u>Am. J. Physiol</u>.

25   274:E627-E633. (1998)), and since it has been
demonstrated herein that α-MSH does not bind the MCH
receptor, it can be inferred that the physiological
antagonism of these two molecules result from the
convergence of signaling pathways activated by distinct

30   receptors.

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EXAMPLE III

Signal transduction pathway of MCH receptor

This example shows that MCH receptor couples to
Gαi- and Gαq-containing G protein signal transduction
5   pathways.

To determine the signaling pathways of SLC-1, a
CHO cell line stably expressing stably SLC-1 was
established as follows. The SLC-1 cDNA subcloned into
pcDNA 3.1 (+) was transfected into CHO dhfr (-) cells by
10 the calcium-phosphate method described in Saito et al.,
J. Neurosci. Res. 48:397-406 (1997), and stable cell
lines were established. To confirm that the plasmid SLC-
1 has been integrated into the CHO genomic DNA, these
cell lines were analyzed by Northern blot and one clone
15 was chosen for further experiments.

In these cells, MCH was able to induce robust
increases in $[Ca^{2+}]_i$ with EC50 of 18.2 ± 4.6 nM (Figure
4C, left). When Gαq/i3 was transiently transfected into
these stable SLC-1-expressing cells, the EC50 for MCH on
20 $[Ca^{2+}]_i$ release was 4.2 ± 0.8 nM (Figure 4C, left), the
same value as that found for the SLC-1-Gαq/i3 transient
cotransfection (Figure 4B).

The effect of MCH on forskolin-stimulated cAMP
accumulation was then examined. SLC-1 expressing cells
25 were plated in 24 well plates and grown to confluency.
After removal of the culture medium, variable amounts of
synthetic MCH in a total volume 0.3 ml of Dulbecco's
modified Eagle's medium [containing 10mM HEPES, 1 μM ·
forskolin, and 2 μM phosphodiesterase inhibitor Ro20-
30 1724] were added and the cells were incubated for 15 min
at 37oC. The medium was aspirated, the cells were

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extracted with 1 ml 70% ethanol, centrifuged to remove
the debris, and the supernatant was lyophilized. cAMP
content was then measured by competitive binding assay
using 125I-cAMP (NEN).

5   In the stably-transfected SLC-1 cells, MCH
potently inhibited forskolin-stimulated cAMP
accumulation, showing that SLC-1 can also induce
inhibition of adenylyl cyclase. In these experiments,
data were normalized to the amounts of cAMP in forskolin-
10 stimulated cells (100%). All incubations were done in
triplicates, with a representative experiment shown in
Figure 4C, right. The EC50 (4.1 ± 1.7 nM) for the cAMP
assay is similar to that found when the Gαq/i3 chimera is
expressed.

15   Together, these data indicate that SLC-1
couples not only to Gαi but also to Gαq, albeit with a
lower affinity. The fact that SLC-1 can couple to
different G proteins indicates that it may activate
different second messenger responses in distinct cellular
20 environments.

EXAMPLE IV
Distribution of MCH receptor

This example shows the expression of the MCH
25 receptor in mammaliam tissues, as determined by Northern
blot analysis and *in situ* hybridization.

The 1.1kb insert of SLC-1 was labeled with
α^{32}P-dCTP and used as a probe in Northern blot analysis.
Northern blots containing 3 µg of poly(A)+ RNA from
30 various rat tissues were hybridized and washed under high

stringency conditions. Blots were exposed to Kodak X-
OMAT film at -80°C with two intensifying screens.

     Northern blot analyses of adult rat tissues
showed that the 2kb SLC-1 mRNA is detected at a high
5 level in the brain, in moderate amounts in the eye and
skeletal muscle and in small amounts in tongue and
pituitary (Figure 5A, top panel). Loading was verified
by hybridization to a G3PDH control probe (Figure 5A,
bottom panel). A possible role for MCH in the eye,
10 skeletal muscle, and tongue has not been thus far
investigated. The existence of SLC-1 in the pituitary
lends support to a neuroendocrine role for MCH (Ludwig et
al., Am. J. Physiol. 274:E627-E633. (1998); Jezova et
al., Endocrinology 130:1024-1029 (1992)).

15   To further localize SLC-1 expression within the
central nervous system, in situ hybridization was
performed using a cRNA probe on rat brain sections. A
0.6 kb BamH1-Xbal fragment of human SLC-1 cDNA was
generated and subcloned into the pBluescript II SK (+)
20 vector. The homology between human and rat sequences is
92% in this fragment. Sense and anti-sense riboprobes
were generated by T7 and T3 RNA polymerases,
respectively, in the presence of 35S-UTP. In situ
hybridization to adult rat whole brain sections was
25 performed as described by Winzer-Serhan et al., Br. Res.
Prots. 3:229-241 (1999). Control hybridization with a
sense strand cRNA produced no specific signal (data not
shown).

     Extensive SLC-1 expression was detected in the
30 hippocampal formation, olfactory regions and the medial
nucleus accumbens (Figure 5B, Panels a,b and c). This
distribution corresponds to the monosynaptic connections

that MCH neurons make with several areas in the brain involved in integrating inputs related to taste and olfaction (Skofitsch et al., Brain Res. Bull 15:635-649 (1985); Bittencourt et al., J. Comp. Neurol. 319:218-245 (1992)). This study reveals a possible role of MCH in olfactory learning and reinforcement mechanisms which are fundamental processes in the regulation of feeding behavior. The presence of SLC-1 in the ventromedial nucleus (VMH) of the hypothalamus, a nucleus known to regulate feeding and metabolism (Figure 5B, Panel c) further supports this hypothesis.

Moderate expression of SLC-1 mRNA was found in the substantia nigra, ventral tegmental area and in the amygdala (Figure 5B, Panels b and c), indicating that MCH may modulate the dopaminergic system. Moderate expression of SLC-1 was also detected in the locus coeruleus (Figure 5B, Panel d) which suggests that MCH may participate in the control of various noradrenergic-modulated responses including vigilance, attention, memory, and sleep.

All journal article, reference and patent citations provided above, in parentheses or otherwise, whether previously stated or not, are incorporated herein by reference in their entirety.

Although the invention has been described with reference to the examples provided above, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

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What is claimed is:

     1.   A method of identifying an MCH receptor agonist or antagonist, comprising:

     (a) contacting an MCH receptor with one or more
5 candidate compounds under conditions wherein said MCH receptor produces a predetermined signal in response to an MCH receptor agonist; and

     (b) identifying a candidate compound that alters production of said predetermined signal, said
10 compound being characterized as an MCH receptor agonist or antagonist.

     2.   The method of claim 1, wherein said predetermined signal is calcium ion influx.

     3.   The method of claim 1, wherein said
15 predetermined signal is cAMP production.

     4.   The method of claim 1, wherein said one or more candidate compounds comprises greater than 10^5 compounds.

     5.   The method of claim 1, wherein said MCH
20 receptor comprises the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, or modification or fragment thereof having MCH receptor activity.

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6.   A method of identifying an MCH receptor
ligand, comprising:

(a) contacting an MCH receptor with one or more
candidate compounds under conditions that allow selective

5   binding between said MCH receptor and an MCH receptor
ligand; and

(b) identifying a compound that selectively
binds said MCH receptor, said compound being
characterized as an MCH receptor ligand.

10   7.   The method of claim 6, wherein said one or
more candidate compounds comprises greater than 10^5
compounds.

8.   The method of claim 6, wherein said MCH
receptor comprises the amino acid sequence of SEQ ID NO:2

15   or SEQ ID NO:4, or modification or fragment thereof
having MCH receptor activity.

9.   A method of identifying an individual
having or susceptible to an MCH receptor-associated
condition, comprising detecting MCH receptor nucleic acid

20   molecule in a sample from said individual, wherein
abnormal structure or expression of said MCH receptor
nucleic acid molecule in said sample indicates that said
individual has or is susceptible to an MCH receptor-
associated condition.

25   10.   The method of claim 9, wherein said MCH
receptor nucleic acid molecule comprises at least a part
of the nucleotide sequence of SEQ ID NO:1.

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11.   The method of claim 9, wherein said MCH receptor-associated condition is a disorder of body weight, mood, memory, learning, sleep, dopaminergic system function, reproduction or growth.

5   12.   A method of identifying an individual having or susceptible to an MCH receptor-associated condition, comprising detecting MCH receptor polypeptide in a sample from said individual, wherein abnormal expression or activity of said MCH receptor polypeptide

10   in said sample indicates that said individual has or is susceptible to an MCH receptor-associated condition.

13.   The method of claim 12, wherein said MCH receptor polypeptide comprises at least a part of the amino acid sequence of SEQ ID NO:2.

15

14.   The method of claim 12, wherein said MCH receptor-associated condition is a disorder of body weight, mood, memory, learning, sleep, dopaminergic system function, reproduction or growth.

20   15.   A signaling composition, comprising:
         (a) a recombinantly expressed MCH receptor; and
         (b) a recombinantly expressed Gα subunit of a G protein.

16.   The composition of claim 15, wherein said

25   MCH receptor comprises the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, or modification or fragment thereof having MCH receptor activity.

17.   The composition of claim 16, wherein said Gα subunit is selected from the group consisting of Gαi,

30   Gαq and chimeric Gα.

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18.   A signaling composition, comprising:

(a)   a recombinantly expressed MCH receptor;

(b) a G protein; and

(c) a calcium indicator.

5   19.   The composition of claim 18, wherein said
MCH receptor comprises the amino acid sequence of SEQ ID
NO:2 or SEQ ID NO:4, or modification or fragment thereof
having MCH receptor activity.

           20.   The composition of claim 18, wherein said
10 G protein comprises an α subunit selected from the group
consisting of Gαi, Gαq and chimeric Gα.

FIG. 1   *1/5*

```
   1 tccaacagac agtttctgtc tctgcttcac tcaagaagcc caggctcaga agataccaat
  61 caaggaaatc cccgctagga agcctggggt agggagagct gctggcttga ccagggcaca
 121 gccggcaaaa gcctctacaa gacagtcacc cacagatatg cccaagaatc agtacacagt
 181 ttccaaccag agatctccaa aatgaaacac tcagggctac acataggaaa agcacgcaca
 241 cacacacaca cacacacaca gacacttact tttgtgtcct tctggctatg ctgacgagtt
 301 ttcctggtga agcccggggc tcacagagta atctctgcag acaactgtgg ttcttgcctc
 361 tggtgcctgc aggaggcagg catgttgtgt ccttccaaga cagatggctc agggcactct
 421 ggtaggattc accaggaaac tcatggagaa gggaaaaggg acaagattag caacagtgaa
 481 gggagggaga atggtgggag aggattccag atgaacggtg ggtcgctgga ggctgagcat
 541 gccagcagga tgtcagttct cagagcaaag cccatgtcaa acagccaacg cttgctcctt
 601 ctgtccccag gatcacctcc tcgcacgggg agcatctcct acatcaacat catcatgcct
 661 tcggtgttcg gcaccatctg cctcctgggc atcatcggga actccacggt catcttcgcg
 721 gtcgtgaaga agtccaagct gcactggtgc aacaacgtcc ccgacatctt catcatcaac
 781 ctctcggtag tagatctcct ctttctcctg ggcatgccct tcatgatcca ccagctcatg
 841 ggcaatgggg tgtggcactt ggggagacc atgtgcaccc tcatcacggc catggatgcc
 901 aatagtcagt tcaccagcac ctacatcctg accgccatgg ccattgaccg ctacctggcc
 961 actgtccacc ccatctcttc cacgaagttc cggaagccct ctgtggccac cctggtgatc
1021 tgcctcctgt gggccctctc cttcatcagc atcaccctg tgtggctgta tgccagactc
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1141 ctctactggt tcaccctgta ccagtttttc ctggcctttg ccctgccttt tgtggtcatc
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1261 cgcagcatcc ggctgcggac aaagagggtg acccgcacag ccatcgccat ctgtctggtc
1321 ttctttgtgt gctgggcacc ctactatgtg ctacagctga cccagttgtc catcagccgc
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1501 tcggtgaagc ctgcagccca ggggcagctt cgcgctgtca gcaacgctca gacggctgac
1561 gaggagagga cagaaagcaa aggcacctga tacttcccct gccaccctgc acacctccaa
1621 gtcagggcac cacaacacgc caccgggaga gatgctgaga aaaacccaag accgctcggg
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1741 tcacacgttg ctggggaggc ctggagtcag gtttgggggtt ttcagatatc agaaatccct
1801 tgggggagca ggatgagacc tttggataga acagaagctg agcaagagaa catgttggtt
1861 tggataaccg gttgcac
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LSFISITPVWLYARLIPFPGGAVGCGIRLPNPDTDLYWFTLYQFFLAFALPFVVITAA
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DEERTESKGT
```

FIG. 2

```
   1   gcaggcgacc tgcaccggct gcatggatct gcaaacctcg ttgctgtcca ctggcccaa
  61   tgccagcaac atctccgatg gccaggataa tctcacattg ccggggtcac ctcctcgcac
 121   agggagtgtc tcctacatca acatcattat gccttccgtg tttggtacca tctgtctcct
 181   gggcatcgtg ggaaactcca cggtcatctt tgctgtggtg aagaagtcca agctacactg
 241   gtgcagcaac gtccccgaca tcttcatcat caacctctct gtggtggatc tgctcttcct
 301   gctgggcatg cctttcatga tccaccagct catggggaac ggcgtctggc actttgggga
 361   aaccatgtgc accctcatca cagccatgga cgccaacagt cagttcacta gcacctacat
 421   cctgactgcc atgaccattg accgctactt ggccaccgtc caccccatct cctccaccaa
 481   gttccggaag ccctccatgg ccaccctggt gatctgcctc ctgtgggcgc tctccttcat
 541   cagtatcacc cctgtgtggc tctacgccag gctcattccc ttcccagggg gtgctgtggg
 601   ctgtggcatc cgcctgccaa acccggacac tgacctctac tggttcactc tgtaccagtt
 661   tttcctggcc tttgcccttc cgtttgtggt cattaccgcc gcatacgtga aatactaca
 721   gcgcatgacg tcttcggtgg ccccagcctc ccaacgcagc atccggcttc ggacaaagag
 781   ggtgacccgc acggccattg ccatctgtct ggtcttcttt gtgtgctggg caccctacta
 841   tgtgctgcag ctgacccagc tgtccatcag ccgcccgacc ctcacgtttg tctacttgta
 901   caacgcggcc atcagcttgg gctatgctaa cagctgcctg aacccctttg tgtacatagt
 961   gctctgtgag acctttcgaa aacgcttggt gttgtcagtg aagcctgcag cccaggggca
1021   gctccgcacg gtcagcaacg ctcagacagc tgatgaggag aggacagaaa gcaaaggcac
1081   ctgacaattc cccagtcgcc tccaagtcag gccaccccat caaaccgtgg ggagagatac
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1201   caaccacatt ccac
```

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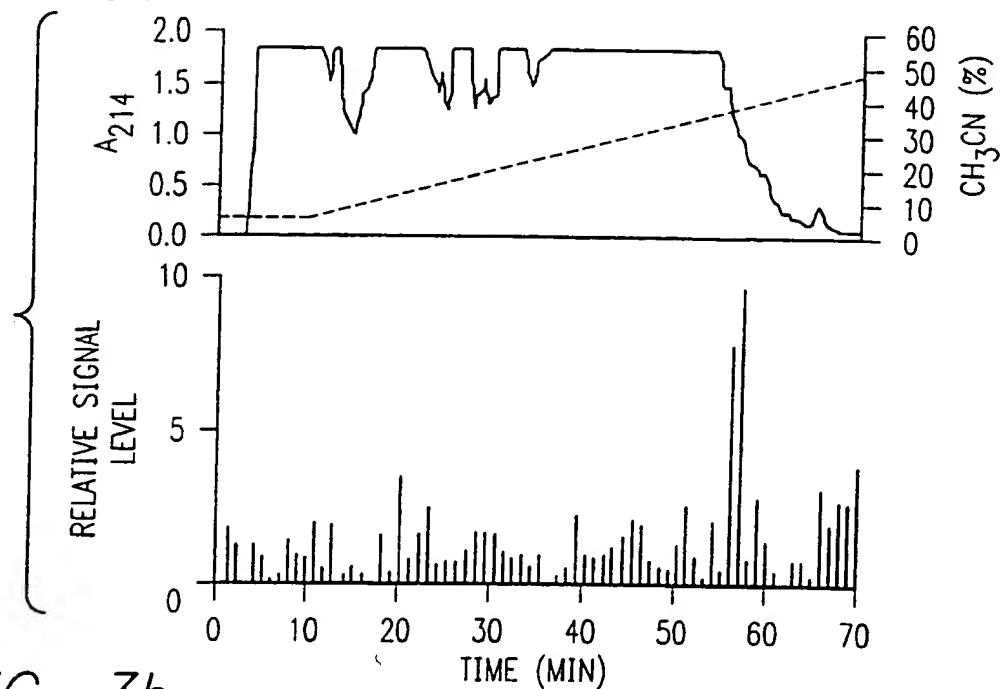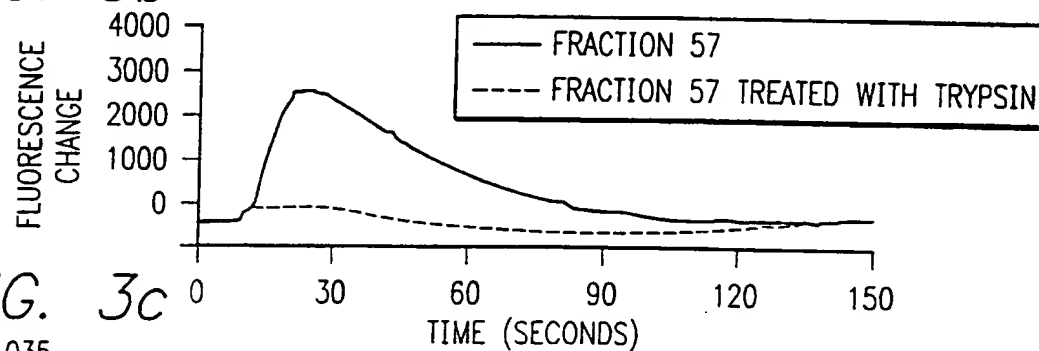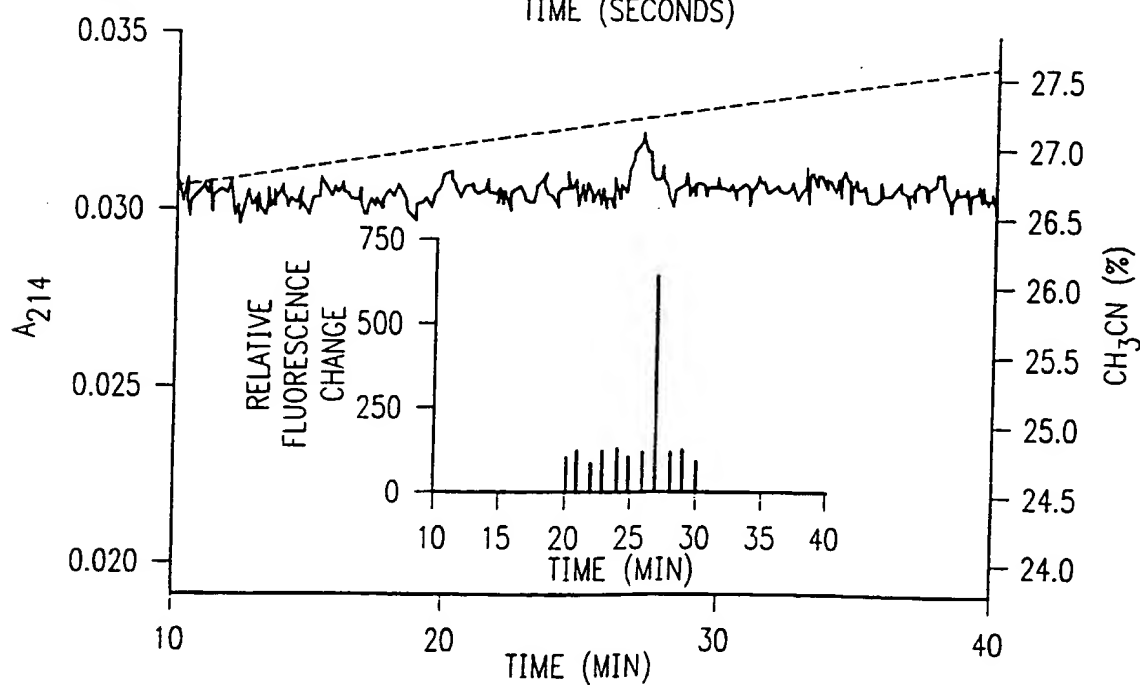
FIG. 3a

FIG. 3b

FRACTION 57
FRACTION 57 TREATED WITH TRYPSIN

FIG. 3c

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FIG. 4a

RAT/HUMAN MCH	DFDMLRCMLGRVYRPCWQV
SALMON MCH	DTMRCMVGRVYRPCWEV
SOMATOSTATIN-14	AGCKNFFWKTFTSC
CORTISTATIN-14	PCKNFFWKTFSSCK

FIG. 4b

Legend:
- ■ MCH
- ▼ SALMON MCH
- ▽ SST-14
- ◇ CS-14
- × α-MSH
- □ NEI
- ● MGOP-14

FIG. 4c

- • SLC-1
- ○ SLC-1 + G αq/i3

- • SLC-1
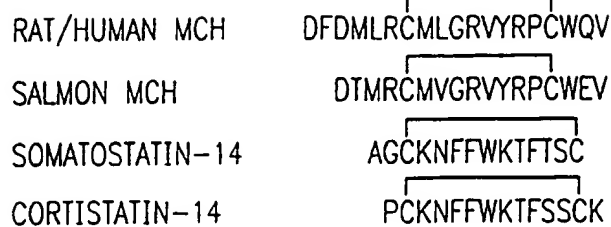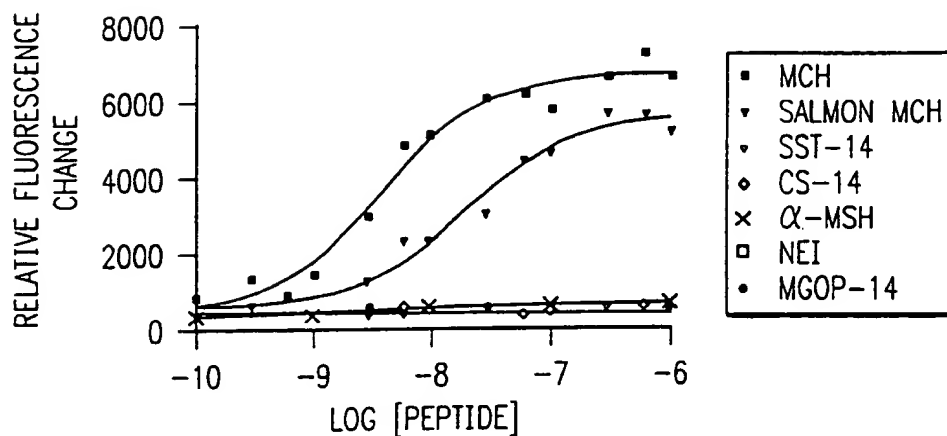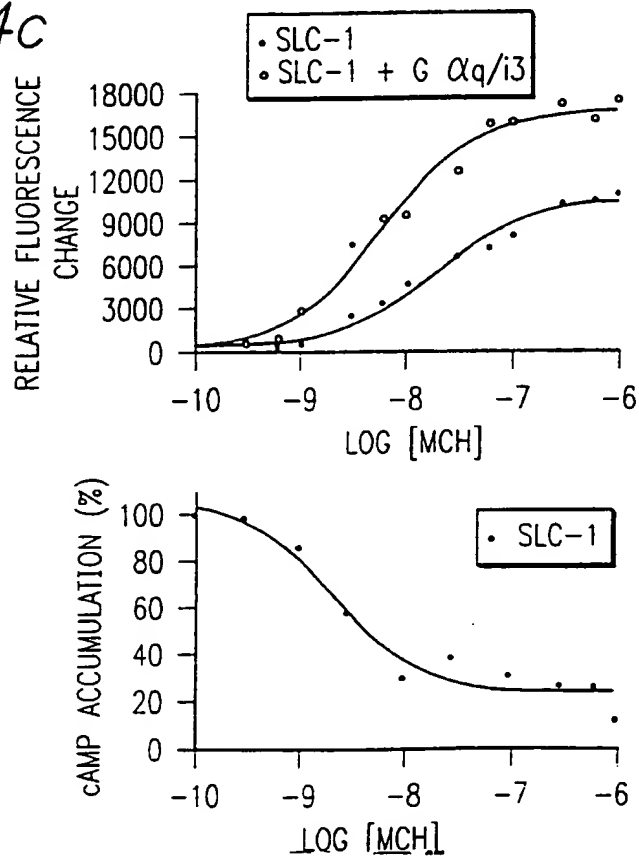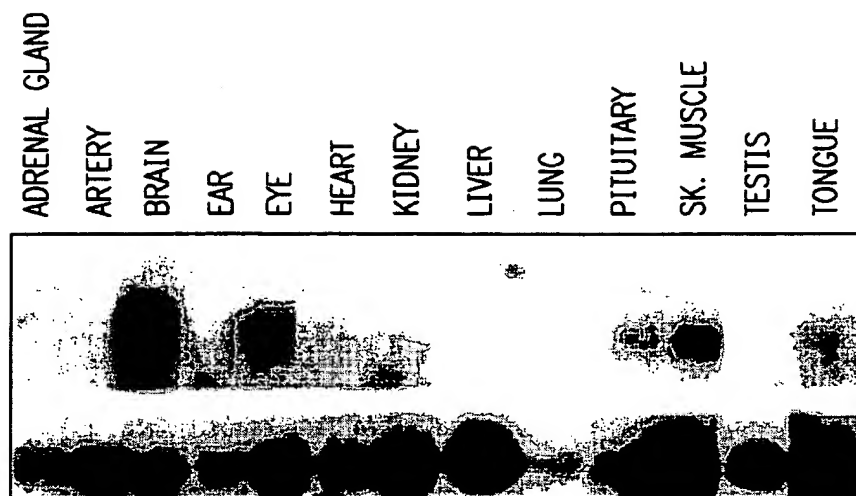
FIG. 5a

FIG. 5b
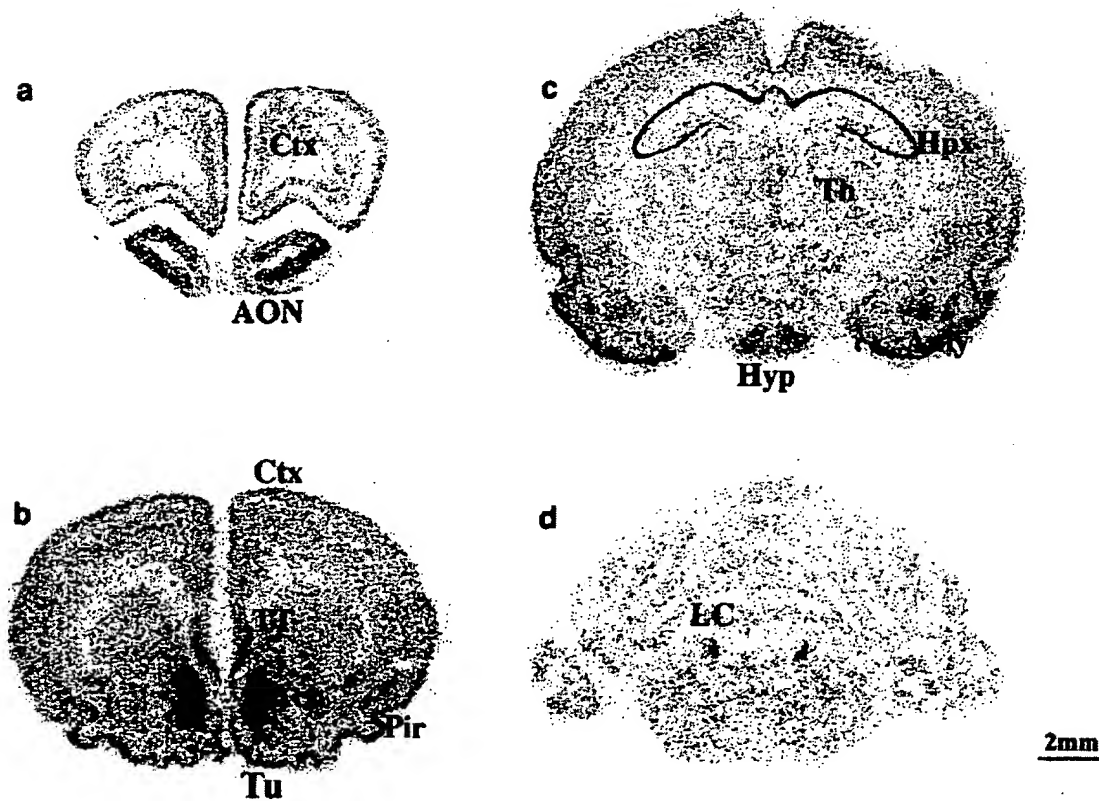
INTERNATIONAL SEARCH REPORT

	International application No. PCT/US00/15503

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C07J 14/72; C07H 21/04; C12P 21/02; G01N 33/53, 31/00
US CL : 530/350; 536/23.5; 435/69.1, 7.1; 436/6

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350; 536/23.5; 435/69.1, 7.1; 436/6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 848 060 A2 (SMITHKLINE BEECHAM CORPORATION) 17 June 1998 (17.06.98), see entire document, especially SEQ ID NOS: 1 and 2, abstract, page 3, lines 47-49, page 12, line 11 to page 13, line 4, page 13, lines 22-53, page 14, lines 30-32, page 15, line 37 to page 16, line 35, and page 20, lines 1-19.	1-14
X	WO 96/18651 A1 (SMITHKLINE BEECHAM CORPORATION) 20 June 1996 (20.06.96), see entire document, especially the abstract, SEQ ID NOS: 1 and 2, page 2, line 14-16 and lines 23-29, page 2, line 40 to page 3, line 10, page 10, line 19 to page 11, line 21, page 13, lines 22-38, and page 16, lines 9-21, and claims 14, 17 and 20.	1-14

☒	Further documents are listed in the continuation of Box C.	☐	See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with on : or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 AUGUST 2000	22 SEP 2000

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer EILEEN B. O'HARA
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P ---- Y, P	US 6,008,012 A (BERGSMA ET AL) 28 December 1999 (28.12.99), see entire document, especially SEQ ID NOS: 1 and 2, the abstract, column 1, lines 63-67, column 2, lines 9-17 and lines 32-43, column 10, lines 43-65, and column 12, lines 45-61.	1-14 ---------- 15-20
X, P ---- Y, P	US 6,033,872 A (BERGSMA ET AL) 07 March 2000 (07.05.00), see abstract, SEQ ID NOS: 1 and 2, column 3, lines 59-63, column 18, lines 27-47, column 19, lines 1-37, column 22, line 45 to column 24, line 6 and column 28, line 36 to column 30, line 2.	1-14 ---------- 15-20
A, E	WO 00/39279 A2 (SYNAPTIC PHARMACEUTICAL CORPORATION) 06 July 2000 (06.07.00), see entire document.	1-4

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

SEQUENCE DATABASES: EST, Issued_Patents_NA, N_Geneseq_36, GenEmbl, strembl12, swiss-prot38, pir64, a-issued, a-geneseq36. SEQ ID NOS searched: 1, 2 and 4.
EAST, STN/CAS, CAPLUS, MEDLINE
search terms: melanin concentrating hormone, receptor, MCH, screen